VOLUME XX FEBRUARY, 1937 NUMBER 2

JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

Contents

A comparison of ten presumptive test media used in the detection of the Escherichia-Aerobacter group in milk. MICHAEL A. FARRELL 67

Mercuric chloride as a preservative for milk samples held for the determination of lactic acid. H. C. TROY and PAUL F. SHARP 77

Variants of streptococcus lactis which do not ferment lactose. E. S. YAWGER, JR., and J. M. SHERMAN 83

A further study of the factor in soybeans affecting the vitamin A value of butter. S. M. HAUGE, J. W. WILBUR and J. H. HILTON 87

A simple plant method of estimating the alkaline constituents of washing powders and washing solutions containing mixed alkalies. LAWRENCE L. LITTLE 93

Fermentative variability among substrains of streptococcus cremoris and streptococcus lactis obtained from pure cultures. J. H. SHERMAN and R. V. HUSSONG 101

The detection and significance of Escherichia-Aerobacter in milk. III. Correlation of total bacterial count and presence of the coli-aerogenes group. M. T. BARTRAM and L. A. BLACK 105

Changes in weight of new born dairy calves as related to the method of feeding. C. L. COLE 113

American Dairy Science Association Announcements 117

Abstracts—Milk and Milk Products 19

THE SCIENCE PRESS PRINTING COMPANY
LIME AND GREEN STREETS, LANCASTER, PA.

Made in United States of America

ALL AMERICA IS BEING EDUCATED TO THE NEED FOR "BOTTLED SUNSHINE"
IRRADIATED VITAMIN D MILK MEANS NEW SALES, NEW PROFITS FOR EVERY MILK DEALER
MOST IRRADIATED MILK IS CP-IRRADIATED
CP IRRADIATOR
in the plant of an enterprising Eastern dealer, who has built a business of more than 80 quarts of Vitamin D Milk per route (name on request). Many dealers report still higher sales.

Under the aggressive promotional work of the Wisconsin Alumni Research Foundation, all America is becoming educated to the importance of Irradiated Vitamin D Milk — not merely as a "must" in winter, but as a year-round habit, and in the Southern states as well as in the North.

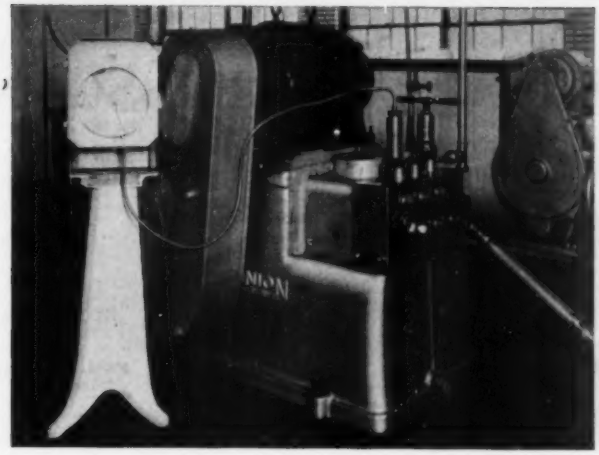
Here is an important new source of high profit sales for milk dealers everywhere — and the Foundation is cooperating not only with nationwide educational work but also with a ready-made campaign of merchandising and direct cooperation with the milk dealer.

Those who have pioneered in the sale of Irradiated Vitamin D Milk find that its extra margin offers a sure and steady source of profit.

From the beginning most Vitamin D Milk has been CP-Irradiated. The Irradiator which CP pioneered embodies every essential of control, convenience, economy and reliability and provides the most economical method of supplying an anti-rachitic food of known potency. CP Irradiators are standard with most progressive milk plants. Investigate the three-fold importance of Irradiated Vitamin D Milk—as an immediate profit-maker, a trade-builder and a sure entree to the best trade in your city. And above all, provide yourself an unfailing source of standard supply—with CP Irradiators. Write for CP Bulletin B-511 or consult the CP salesman.

CP 50th ANNIVERSARY 1887 1937
Creamery Package
THE CREAMERY PACKAGE MFG. COMPANY
1243 West Washington Boulevard, Chicago, Illinois *Also Branches in 19 principal cities*
CREAMERY PACKAGE MFG. CO. OF CANADA, LTD. THE CREAMERY PACKAGE MFG. CO. LTD.
267 King St. West, Toronto, Ont., Can. Avery House, Clerkenwell Green, London, E. C. 1, Eng.

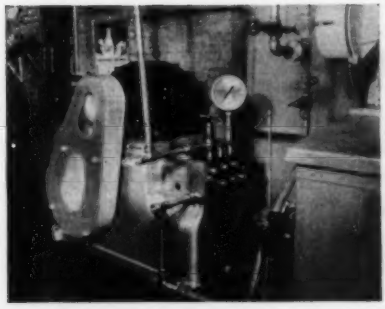
Entered as second-class mail matter April 13, 1934, at the Post Office at Lancaster, Pa.
Under the Act of March 3, 1879.
Copyrighted, 1932, by the American Dairy Science Association.

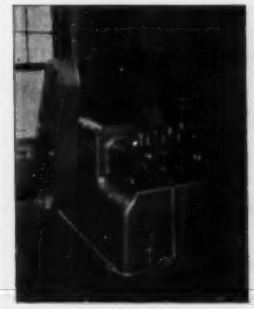


No. 1036 Cherry-Burrell Sanitary Viscolizer in the plant of Ewing Von-Allman Company, Louisville, Ky. This Viscolizer is employed in the processing of ice cream mix and condensed milk.

The

NEW AND TRULY SANITARY VISCOLIZER





Above is a No. 536 Cherry-Burrell Sanitary Viscolizer employed in processing ice cream mix and condensed milk for the Goshen Milk Company of Goshen, Indiana.

In the lower photograph is a No. 536 on ice cream mix in the plant of the Malvern Dairy, Washington, D. C.

Swings into its Stride!

IT IS no exaggeration to say that the acceptance of the new CHERRY-BURRELL VISCOLIZER has been both immediate and nation-wide. Already you see them everywhere, in plants of all types, and on all types of work.

The viscolization of milk, ice cream mix, condensed milk and a host of other liquid and semi-liquid food products now becomes truly practical and desirable.

This NEW CHERRY-BURRELL VISCOLIZER is truly sanitary . . . as easy to clean and keep clean as any other of the simplest items of equipment, as you will at once grasp when you note these new and definitely superior features which have been worked out for you:

1. Rustless Stainless Steel (18-8) Cylinder Block. 2. Removable one-piece packing. 3. No threads on the inside of the cylinder. 4. All ports and chambers readily accessible. 5. Specially hardened stainless plungers which are easily removed. 6. Cylinder that can be completely taken apart and reassembled in less than a half hour. 7. Cylinder that can be conveniently removed from machine. 8. Sanitary packing boxes that can be easily washed and sterilized. 9. All passages highly machined to a smooth finish.

APPROVED BY HEALTH OFFICIALS

This New Viscolizer has been approved by some of the most exacting health officials in the country. It is available in the following capacities: 100, 200, 300, 500, 800, 1,000 gallons per hour. Send for literature illustrating and describing this latest development of our engineers.

CHERRY-BURRELL CORPORATION 427 W. RANDOLPH ST., CHICAGO

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. DAHLBERG, Editor
Geneva, N. Y.

Associate Editors

L. S. PALMER, St. Paul, Minn.
C. W. LARSON, Buffalo, N. Y.
H. A. RUEHE, Urbana, Ill.
E. G. HASTINGS, Madison, Wis.
E. B. MEIGS, Beltsville, Md.
PAUL F. SHARP, Ithaca, N. Y.
O. F. HUNZIKER, Chicago, Ill.
L. A. ROGERS, Washington, D. C.
C. E. GRAY, San Francisco, Cal.

Committee on Journal Management

O. F. HUNZIKER, Chairman
Chicago, Ill.

R. B. STOLTZ, Columbus, O.
A. A. BORLAND, State College, Pa.

The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to A. C. Dahlberg, New York Agricultural Experiment Station, Geneva, New York. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

Subscriptions. Price; $6.00 per volume in United States, Canada and countries within the postal union; $6.50 in countries outside the postal union. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume unless otherwise stated. Renewals should be made promptly to avoid a break in the series.

Subscriptions and Advertising should be mailed direct to the Science Press Printing Company, Lime and Green Streets, Lancaster, Pennsylvania.

All subscriptions from the British Isles and British Empire (with the exception of Canada) should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W.C.2, England.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio. The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

GLASS

TELLS THE TRUTH ABOUT MILK . . .

Because milk contributes so importantly to the health of mankind, the truth, and nothing but the truth, about its richness, wholesomeness and purity must be clearly and instantly revealed to the purchaser. And that is precisely what the glass milk bottle, and only the glass milk bottle, does. Depend upon glass to carry milk and other dairy products to customers with the assurance and honesty that only glass can convey. Owens-Illinois Glass Company, Dairy Container Division, Toledo.

OWENS-ILLINOIS
Glass Containers

Your advertisement is being read in every State and in 25 Foreign Countries

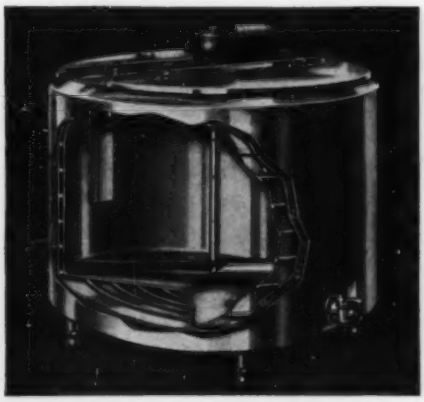
An ALL-PURPOSE UNIT

THE Mojonnier COUNTER-CURRENT STAINLESS STEEL PASTEURIZER

The heating or cooling water in this new pasteurizer is circulated through a spiral construction in the jacket of the vat. The long water travel, at a relatively high velocity, guarantees exceptionally fast heat transfer. Consequently, these pasteurizers are suitable not only for heating but also for cooling directly in the vat. On actual installations users state it is the fastest and most efficient pasteurizer they have ever used for this purpose.

The Mojonnier Pasteurizer offers the important advantage of easy cleaning. There are no coils, flanges or stuffing boxes used in the construction of the vat.

These all-purpose pasteurizers are made in all capacities from 50 gallons up. Several applications are as follows:



1 **For pasteurizing milk.**

2 **For processing cream, buttermilk, and starter, where cooling is done directly in the vat.**

3 **For making ice cream mix.**

4 **For use as a combination hot well and cooler for viscous products in conjunction with the operation of stainless steel vacuum condensing equipment.**

Colleges and experimental stations will find the smaller sizes especially suited to their needs. All inquiries will be given prompt attention.

MOJONNIER BROS. CO.
4601 W. OHIO ST., CHICAGO, ILL.

Cut-Away View of Mojonnier Counter Current Pasteurizer showing spiral construction in the wall of the vat. Note type of paddle agitator which will not incorporate air.

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

VOLUME XX FEBRUARY, 1937 NUMBER 2

A COMPARISON OF TEN PRESUMPTIVE TEST MEDIA USED IN THE DETECTION OF THE ESCHERICHIA–AEROBACTER GROUP IN MILK*

MICHAEL A. FARRELL
Division of Bacteriology, The Pennsylvania State College

The importance and significance of the colon group of organisms in raw milk is a disputed question among milk sanitarians (1, 2). It is generally conceded, however, that the test for these organisms offers an added safety precaution in controlling the efficiency of pasteurization (3, 4). "Standard Methods of Milk Analysis" (5) has recently included a tentative procedure for the detection of the *Escherichia-Aerobacter* group of organisms in milk. It advocates the use of 2.0 per cent brilliant green lactose bile as the presumptive test medium. In England the Ministry of Health (6) recommends a combined colony and coliform count on samples of certified and Grade A pasteurized, Grade A tuberculin tested, and Grade A milk. It suggests the use of MacConkey's lactose peptone bile broth as a presumptive test medium, for the determination of colon group members. The question has often arisen since these two media were primarily developed for the detection of these organisms in water, whether they are the best presumptive test media for the detection of *Escherichia-Aerobacter* organisms in milk (7). This study was undertaken with the object of answering this inquiry.

PROCEDURE

Ten presumptive test media which have been proposed for use in the detection of the *Escherichia-Aerobacter* group of organisms in water and milk have been included in this study. The names of these media and the abbreviations employed in this paper to facilitate the presentation of tabular material are listed below:

1. Lactose broth—S.L. (8)
2. Buffered lactose broth—B.L. (9)
3. Brilliant green lactose bile broth 2.0%—B.G. (10)
4. Fuchsin lactose broth—F.L. (11)

Received for publication August 10, 1936.

* Authorized for publication on February 18, 1936, as paper No. 725 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

5. Methylene blue-brom-cresol purple broth—M.B. (12)
6. Crystal violet lactose broth—C.V.L. (13)
7. Formate ricinoleate broth—F.R. (14)
8. Gentian violet lactose broth—G.V. (3)
9. MacConkey's peptone lactose bile broth—P.L.B. (15)
10. Trypaflavine broth—Tryp. (16)

The first seven media were obtained in dehydrated form from the Difco Laboratories. These media were specially prepared for the "Committee on Standard Methods of Water Analysis," and were verified as being up to specifications of their proponents.[1] The eighth medium is included because it was one of the first media recommended for the detection of *Escherichia-Aerobacter* members in milk. The remaining two media were included for comparison because of their general acceptance as presumptive test media in England and Germany. MacConkey's medium was made according to his formula (15) and trypaflavine broth after the directions of Ruhmekorf (16). Three dilutions, 1.0 cc., 0.1 cc. and 0.01 cc., of each milk sample were employed and five tubes of each medium were planted from each of the three dilutions. Dilutions were prepared by transferring eleven cubic centimeters from the lower dilution into 99 cc. of sterile distilled water. These inoculated tubes were incubated for 48 hours at 37° C., after which interval they were examined for gas production. One tube of each medium showing gas formation (the highest dilution) was separately carried through the confirmatory and completed tests according to "Standard Methods of Water Analysis" (8).

DATA

The results of planting sixty-six samples of raw and certified milk into ten presumptive test media for the purpose of detecting the *Escherichia-Aerobacter* group of organisms is shown in the table which follows.

Table 1 shows the actual number of milk samples in which *Escherichia-Aerobacter* organisms were found to be present when sixty-six milk samples were planted into the ten presumptive test media and followed by the usual confirmatory tests. The average number of doubtful and positive presumptive tests is approximately forty-three, whereas the average number of tests which on completion were positive was approximately twenty-eight. The third horizontal column shows the percentage of presumptive tests which were positive upon confirmation and gives some idea of the time, labor and materials wasted in confirming the presumptive evidence observed when certain of these media were employed. Three of these ten media, brilliant green lactose bile broth, fuchsin lactose broth and methylene blue-brom cresol purple broth showed the largest number, thirty-five, of positive com-

[1] The proponent of each medium was asked to make comparisons of his own and the dehydrated product prepared by the Difco Laboratories in order to ascertain that the dehydrated product met the proponents' specifications.

TABLE 1

The efficiency of ten presumptive test media in the detection of the Escherichia-Aerobacter group in sixty-six raw milk samples

	S.I.	B.L.	B.G.	F.L.	M.B.	C.V.L.	F.R.	P.L.B.	TRYP.	G.V.*
Number of Doubtful and Positive Presumptive Tests	35	42	44	45	43	40	61	47	31	34
Number of Positive Completed Tests	24	24	35	35	35	25	32	29	23	17
Per cent of Doubtful & Positive Presumptive Tests which upon Completion were Positive	68.6	57.1	79.5	77.7	81.9	62.5	50.8	61.7	74.2	50.0
Per cent of all Samples Positive	36.3	36.3	53.0	53.0	53.0	37.9	48.5	43.9	34.5	36.2

* Only forty-seven samples were run on this medium.

pleted tests for the *Escherichia-Aerobacter* group of bacteria. In terms of percentage of all samples positive, each of these three media showed an efficiency of *fifty-three per cent* in the detection of the *Escherichia-Aerobacter* group of bacteria in milk. The remaining seven media were definitely less efficient in the detection of this group of bacteria when both the numbers of presumptive tests confirmed and the percentage of all samples positive were considered.

SIGNIFICANCE OF THE "MOST PROBABLE NUMBER" INDEX

The tentative procedure for the detection of the colon group of bacteria as outlined in the sixth edition of "Standard Methods of Milk Analysis" (5) also includes information relative to the convenience of enumerating the "Most Probable Number" (M.P.N.) of *Escherichia-Aerobacter* organisms per 100 cubic centimeters of milk. The question naturally arises since these presumptive test media were not developed essentially for the detection of the colon group of organisms in milk, to what extent in each of these presumptive test media was the M.P.N. value found a true measure of the "coli" density in these milk samples. The data obtained in this study offers an answer to this inquiry.

The data obtained in the "presumptive test" when these sixty-six milk samples were planted in multiple portions (5 tubes)[2] for each of the three dilutions employed, offered a comparison not only of the presence or absence

[2] It is realized that the accuracy of a computed M.P.N. value when multiple portions of only five tubes are used as recommended in "Standard Methods of Milk Analysis" (5) is open to criticism. It is believed, however, that this multiple number offers a basis of comparison for these ten media as they are commonly used in routine work.

of *Escherichia-Aerobacter* organisms in these milk samples but also showed the density of the colon group of bacteria. Assuming that the presumptive test medium which shows the largest number of positive presumptive tests followed by the greatest percentage confirmation to be the most efficient medium in the detection of *Escherichia-Aerobacter* members in milk, it was of interest to correlate the "coli" density as indicated by these M.P.N. values with the actual presence or absence of colon members as found by the results of confirmatory procedure. McCrady's tables as found in "Standard Methods of Milk Analysis" (5) were employed to obtain these M.P.N. values. The following table in condensed form shows the M.P.N. values obtained for each of the sixty-six milk samples on each of these ten presumptive test media.

Table 2 shows the M.P.N. values and their frequency occurrence when these sixty-six milk samples were planted into these ten presumptive test media. The first horizontal column shows the number of milk samples, using the different media, found to have no *Escherichia-Aerobacter* group members present in the three dilutions employed. Brilliant green lactose bile broth showed gas formation in all but twenty-two samples, while formate-ricinoleate broth and trypaflavine broth were the two extremes and showed gas formation in all but five and thirty-six of the sixty-six milk samples respectively. The average M.P.N. values for these media based on the number of samples showing gas formation in each medium is shown at the bottom of the table. These averages show that formate ricinoleate broth has the highest average "Most Probable Number" with ten colon or other gas forming bacteria per cubic centimeter of milk. Brilliant green lactose bile broth, buffered lactose broth and crystal violet lactose broth, lead the remaining media with values of 5.9, 5.3 and 4.6 organisms per cubic centimeter respectively, while the other media all have an average M.P.N. of less than four organisms per cubic centimeter.

To ascertain the accuracy of the presumption that these M.P.N. values were the result of gas production by *Escherichia-Aerobacter* members only, the first and third horizontal columns in table 1 should be examined. Those media such as formate ricinoleate broth, buffered lactose broth and crystal violet lactose broth which showed gas production in sixty-one, forty-two and forty milk samples respectively were found upon confirmatory procedure to contain members of the *Escherichia-Aerobacter* group in only fifty-one per cent of the formate ricinoleate tubes, fifty-seven per cent of the buffered lactose tubes and sixty-three per cent of the crystal violet lactose samples showing gas production. The high M.P.N. values shown for these media in table 2, therefore, does not indicate a greater ability to detect members of the *Escherichia-Aerobacter* group. A possible interpretation of this result is that these media showing high M.P.N. values with lower percentages of doubtful and positive presumptives confirmed, were fermented by bacteria

TABLE 2

*The frequency occurrence of the most probable number of Escherichia-Aerobacter organisms in sixty-six milk samples in ten presumptive test media**

SIGNIFICANT NO.	M.P.N./CC.	S.L.	B.L.	B.G.	F.L.	M.B.	C.V.L.	F.R.	P.L.B.	TRYP.	G.V.**
0–0–0	0	31	24	22	21	23	26	5	19	35	13
0–0–1 to 1–4–0	0.2 to 1.1	15	18	8	17	15	15	16	16	14	15
2–0–0 to 2–4–0	0.5 to 1.4	5	5	9	10	11	9	5	6	6	7
3–0–0 to 3–5–0	0.8 to 2.5	8	6	9	5	7	5	7	9	1	4
4–0–0 to 4–5–1	1.3 to 5.0	3	7	7	5	1	3	7	5	4	2
5–0–0 to 5–5–5	2.5 to 180.0 +	4	6	11	8	9	8	26	11	6	6
Total Most Probable Number		100.1	223.9	260.0	168.7	171.4	182.3	611.7	109.4	36.9	98.8
Average Most Probable Number Per cc.		2.86	5.33	5.91	3.75	3.99	4.55	10.03	2.33	1.19	2.91

* This table has been condensed primarily to save space. A listing of the significant numbers separately would have necessitated a two-page table.

** Only forty-seven milk samples were run on this medium.

other than the *Escherichia-Aerobacter* group, or that the colon organisms were eventually overgrown by other species of bacteria present in the milk. Brilliant green lactose bile broth, fuchsin lactose broth and methylene blue-brom cresol purple broth with lower M.P.N. values, showed gas production in forty-four, forty-five and forty-three milk samples respectively. The subsequent confirmatory procedure indicated the presence of *Escherichia-Aerobacter* members in eighty per cent of the brilliant green, seventy-eight per cent of the fuchsin and eighty-two per cent of the methylene blue-brom cresol purple samples showing gas production in the presumptive test.

ESCHERICHIA-AEROBACTER CULTURES ISOLATED

The 319 cultures obtained from well isolated colonies on eosine methylene blue plates were found to contain 140 cultures of the *Escherichia-Aerobacter* group. These 140 cultures were tentatively determined to belong to the genus by the production of gas in lactose broth, their gram-negative property and their reaction combinations by the formation of indol, and acetyl methyl-carbinol; growth in citrate medium and the methyl red test. They were then identified within the genus by using the necessary tests for keying out the species in Bergey's (17) Manual. Seventy-four cultures or fifty-three per cent were found to belong in the genus *Escherichia* and fifty-six or forty per cent in the genus *Aerobacter*. The remaining ten cultures comprising seven per cent of the total were intermediate cultures. These percentages are slightly higher than those found by Yale (18) for raw milk.

DISCUSSION

The question concerning the efficiency of brilliant green lactose bile broth as well as other media used in the detection of the *Escherichia-Aerobacter* group of organisms in milk is a pertinent one in view of the tentative inclusion of a method in "Standard Methods of Milk Analysis" (5) for the detection of these organisms in milk and other dairy products. In a fundamental study (20, 21) the ability of small numbers of the *Escherichia-Aerobacter* group to grow in various presumptive test media has been determined. Brilliant green lactose bile was found to have a desirability rating of fifth and fourth respectively.

Most of these presumptive test media were developed with respect to their dye content, percentage of ox-bile or bile salts, etc., with the object of inhibiting certain organisms responsible for false presumptive tests in water analysis. The use of these presumptive test media for the detection of colon organisms in milk with the expectation that the inhibiting agents present will prevent false presumptive tests to the same degree that they do in water bacteriology is an erroneous supposition, since it is well known that the presence of organic matter, which in milk is present in the form of proteins,

carbohydrates and lipoids very likely greatly decreased the concentration of these inhibitory substances through adsorption. With little or no inhibiting ingredients active in these media their ability to act as selective media for the detection of *Escherichia-Aerobacter* members in milk appears doubtful and the resulting development of bacteria would depend on the initial numbers of the various species present, their growth rates, and other factors.

Brilliant green lactose bile broth as recommended by "Standard Methods of Milk Analysis" (1934) was found in this study to be one of the three most efficient media in the detection of *Escherichia-Aerobacter* organisms in milk. The writer did not find the high percentage of confirmations for brilliant green lactose bile as were obtained by McCrady and Archambault (19) even when more than one colony was chosen for confirmation. MacConkey's broth and trypaflavine broth as recommended in England and Germany do not appear from this work to be especially efficient in the detection of the *Escherichia-Aerobacter* group of bacteria in milk.

The use of a liquid medium and of a multiple number of tubes of each dilution for the enumeration of small numbers of *Escherichia-Aerobacter* organisms in milk is believed to be more accurate than when a solid-liquefiable culture medium is used (19). The utilization of this multiple tube inoculation to determine the density of *Escherichia-Aerobacter* organisms in milk was shown to be an erroneous procedure in this study involving a thousand tubes of each medium, where it was found that certain of those media having the highest "Most Probable Number" index are apparently permitting the growth of organisms other than the *Escherichia-Aerobacter* group.

In view of the above findings (1) that the majority of these media were not developed for the detection of *Escherichia-Aerobacter* member in milk; (2) that certain of these presumptive test media are fermented with gas production by bacteria other than the colon group of organisms and (3) that brilliant green lactose bile broth is no more selective for the *Escherichia-Aerobacter* group of bacteria than two other presumptive test medium, the question arises—Do we possess an efficient presumptive test medium for the detection of the *Escherichia-Aerobacter* group of bacteria in milk? From the evidence obtained in these experiments the writer is inclined to believe we do not. In addition to those conditions mentioned above this belief is based on the findings that the three media found in this study to be superior in the detection of the *Escherichia-Aerobacter* group were often irregular in detecting colon organisms in the same sample. These three media also showed only eighty per cent confirmation of doubtful and positive presumptive tests. This indicates that even with these better media there were still twenty per cent of false presumptive tests. If the confirmation of every fifth doubtful presumptive test is going to involve a waste of time and materials it appears to the writer that there is an opportunity for the develop-

ment of a more efficient presumptive test medium for detecting this group of bacteria in milk.

The writer wishes to thank Mr. T. Polansky for his assistance in the course of this work.

SUMMARY

1. Brilliant green lactose bile broth, fuchsin lactose broth and methylene blue-brom cresol purple broth were found to be equally efficient in the detection of *Escherichia-Aerobacter* members in sixty-six raw milk samples.

2. The remaining seven media appear definitely less efficient in the detection of these organisms.

3. The use of the "Most Probable Number" index as a measure of the density of the *Escherichia-Aerobacter* group in milk was found to be very inaccurate when certain of these presumptive test media were employed.

4. The question is raised whether we possess a reliable presumptive test medium for the detection of these organisms in milk.

REFERENCES

(1) Sherman, J. M., and Wing, H. U. The Significance of Colon Bacteria in Milk, with Special Reference to Standards. Journal of Dairy Science 16: 165–75. 1935.

(2) Kline, E. K. The Colon Group of Bacteria in Milk. Int. Assoc. Dairy & Milk Insp. Ann. Report 19: 68–85. 1930.

(3) Swenarten, J. C. Can B. Coli be Used as an Index of the Proper Pasteurization of Milk. Jour. Bact. 13: 419–29. 1927.

(4) McGrady, M. H., and Langevin, Em. The Coli-Aerogenes Determination in Pasteurized Control. Journal of Dairy Science 15: 321–29. 1932.

(5) Standard Methods of Milk Analysis. Amer. Pub. Health Assoc., 450 Seventh Ave., New York City, N. Y. 1934.

(6) Ministry of Health. Memo. 139. Foods, His Majesty's Stationery Office, Adastral House, Kingsway, London—W. C. 2, England. 1934.

(7) Breed, R. S. Discussion of C. E. A. Winslow's Paper, "Standard Methods and New Procedures for the Isolation of Colon Bacilli from Water." Am. Jour. Pub. Health 24: 469. 1934.

(8) Standard Methods of Water Analysis. Amer. Pub. Health Assoc., 450 Seventh Ave., New York City, N. Y. 1933.

(9) Ruchhoft, C. C., Kallas, J. G., and Chinn, Ben. Gas Production and pH Determinations of Coli-aerogenes Cultures in Sugar Broths. Jour. Am. W. W. Assoc. 23: 565–81. 1931.

(10) Jordan, H. E. Brilliant Green Bile for the Detection of the Colon-Aerogenes Group. Jour. Am. W. W. Assoc. 18: 337–46. 1927.

(11) Ritter, C. The Presumptive Test in Water Analysis. Jour. Am. W. W. Assoc. 24: 413–24. 1932.

(12) Dominick, J. R., and Lauter, C. J. Methylene Blue and Brom Cresol Purple in Differentiating Bacteria of the Colon Aerogenes Group. Jour. Am. W. W. Assoc. 21: 1067–75. 1929.

(13) Salle, A. J. A System for the Bacteriological Analysis of Water. Jour. Bact. 20: 381–406. 1930.

(14) STARK, C. N., AND ENGLAND, C. W. Formate Ricinoleate Broth—A New Medium for the Detection of Colon Organisms in Water and Milk. Jour. Bact. 29: 26–27. 1933.

(15) MACCONKEY, A. T. Bile Salt Media and their Advantages in Some Bacteriological Examinations. Jour. Hyg. 8: 322–34. 1908.

(16) Ruhmekorf. Über die Bedeutung des Gesamtkeimgehaltes und des Colititers in der Milch. Der Coligehalt von Leipziger Milchsorten. Milchw. Forsch, 11: 600. 1931.

(17) BERGEY, D. Williams & Wilkins, Baltimore, Md. Determinative Bacteriology. 1934.

(18) YALE, M. W. The Escherichia-Aerobacter Group of Bacteria in Dairy Products. JOURNAL OF DAIRY SCIENCE 16: 481–94. 1933.

(19) MCCRADY, M. H., AND ARCHAMBAULT. Examining Dairy Products for Members of the Escherichia-Aerobacter Group. Amer. Jour. Pub. Health 24: 122–28. 1934.

(20) NORTON, J. F., AND RUCHHOFT, C. C. A Study of Selective Media for Coli-Aerogenes Isolation. Jour. Am. W. W. Assoc. 27: 1134–42. 1935.

(21) FARRELL, M. A. A Comparative Study of Nine Presumptive Test Media. Jour. Am. W. W. Assoc. 28: 611–23. 1936.

MERCURIC CHLORIDE AS A PRESERVATIVE FOR MILK SAMPLES HELD FOR THE DETERMINATION OF LACTIC ACID

H. C. TROY AND PAUL F. SHARP
Department of Dairy Industry, Cornell University, Ithaca, New York

Methods for the quantitative determination of lactic acid in dairy products and directions for preserving milk samples for such determinations, were recently published by Troy and Sharp (2) (3). It was stated that "mercuric chloride in a concentration of about 0.2 to 0.5 per cent seems to be a satisfactory preservative for periods of a month or less if the milk is kept in the dark at 20° C. or below."

A number of samples of milk received for lactic acid determination were found to increase slowly in lactic acid value upon holding. These samples contained a black deposit of reduced mercury. This experience caused us to reinvestigate the use of mercuric chloride as a preservative.

Aliquots of sweet and partially soured whole milk were preserved with different amounts of mercuric chloride. A black precipitate of reduced mercury formed if somewhat less than 0.5 per cent of mercuric chloride was added. The tendency of the mercuric chloride to reduce increased with the decrease in the amount added. The samples were held at ordinary laboratory temperatures in clear glass bottles in diffused daylight.

The lactic acid values obtained after various intervals of time are given in table 1. It is evident that 0.2 per cent of mercuric chloride represents the lower limit of concentration for preservation of samples for one month under these conditions, and that 0.5 per cent would allow a margin of safety. The presence of a dark precipitate of reduced mercury may be used as an indication that insufficient mercuric chloride was added for the optimum preservation of the sample for the lactic acid determination.

No bacteria were found in the samples when they were plated at the end of 12 weeks by Pauline Stark of the department of bacteriology. At the end of the experiments reported in tables 1 and 2 the samples were streaked in duplicate on nutrient agar and on milk agar slopes. The presence of microorganisms was evidenced in the samples containing 0.02 and 0.05 per cent of mercuric chloride, but not in any of the samples containing 0.2 per cent or more.

After holding for long periods of time blue filtrates were obtained from the precipitation with copper sulfate and lime suspension in the clarification of the milk for the determination of lactic acid. The blueness of the filtrate was inversely proportional to the concentration of mercuric chloride. Corresponding with the blueness of the filtrates, proportionately greater diffi-

Received for publication August 29, 1936.

TABLE 1

Effect of increasing amounts of mercuric chloride in preserving milk for the determination of lactic acid

MERCURIC CHLORIDE	LACTIC ACID VALUES AFTER HOLDING							PROTEIN NOT PRECIPITATED BY $Cu(OH)_2$ AFTER HOLDING			MICROORGANISMS	
	0	2 weeks	4 weeks	6 weeks	12 weeks	18 weeks	28 weeks	12 weeks	18 weeks	28 weeks	12 weeks	28 weeks
%	%	%	%	%	%	%	%	%	%	%		
Sweet whole milk—unheated												
.05	.000	.000	.003	.010	.014	.024	.....	.35	.95	.....	absent	present
.20	.000	.000	.000	.008	.011	.011	.021	.17	.24	.30	absent	absent
.50	.000	.000	.000	.001	.001	.002	.013	.16	.22	.23	absent	absent
2.00	.000	.000	.000	.000	.001	.001	.002	.10	.10	.16	absent	absent
Partially soured whole milk—unheated												
.05	.141	.142	.149	.158	.166	.166	.....	.95	1.97	.....	absent	present
.20	.141	.141	.144	.153	.158	.165	.204	.51	.66	1.13	absent	absent
.50	.141	.140	.142	.146	.150	.160	.181	.23	.31	.50	absent	absent
2.00	.141	.140	.142	.150	.148	.158	.178	.14	.18	.21	absent	absent

culty with fading endpoints in the acetaldehyde titration was encountered. In using $Cu(OH)_2$ for protein precipitation as recommended in the method previously (2) described, it was found that blue filtrates indicated the presence of protein split products. The nitrogen content of the filtrates of the samples represented in table 1 was found to increase progressively with time and with a decrease in mercuric chloride. The increase in lactic acid value is related to the increase in protein hydrolysis. Probably little or no increase in lactic acid occurred, the apparent increase being due to the interference of protein split products which were not removed by the $Cu(OH)_2$.

The results in table 1 show that protein hydrolysis was more marked in the partially soured samples. Since the milk was not heated, the hydrolysis might be due to proteolytic enzymes. In addition to the natural proteolytic enzymes of the milk, the partially soured samples contained enzymes derived from the bacteria. Increasing amounts of mercuric chloride exert a progressively retarding effect on proteolytic enzymes.

Table 2 presents the results obtained when the experiment was repeated, but in this case each sample was divided into two parts and one part was heated for 5 minutes at 80° C., to destroy the enzyme activity of the milk and the bacteria. At the end of 4 weeks the soluble protein had increased more in the unheated partially soured samples than in the unheated sweet samples. By the end of 12 weeks a definite increase had taken place in all samples. The increase in soluble protein may be attributed to enzyme action and advantage may be taken of mercuric chloride as a preservative for studying proteolytic enzyme activity in milk under various conditions. The proteolysis which occurred in the heated samples indicates that at least some hydrolysis took place without the aid of enzymes and therefore an alternate explanation is possible.

Proteins are slowly denaturized and hydrolized on long standing in contact with water, particularly if they are not near their isoelectric points. Hydrolysis is more rapid if the protein is in solution. Carpenter (1) has very clearly shown the effect of time, temperature and pH on the hydrolysis of casein. Thus the hydrolysis shown in tables 1 and 2 may not be due to enzymes, but to the natural hydrolytic action of the water. Heavy metal salts inactivate many enzymes and may have inactivated the proteolytic enzymes in this case. The rate of natural hydrolysis would be expected to be inversely related to the insolubilizing effect of heat and increasing concentration of mercuric chloride. The preliminary slight proteolytic action of the bacteria in the partially soured samples might prepare the proteins for a more rapid natural hydrolysis. The marked proteolysis of the unheated samples containing the smaller amounts of mercuric chloride was probably due to both enzymes and the natural hydrolytic effect of the water.

The cans of milk which were rejected one morning at the receiving platform of a country milk plant because of undesirable odors were sampled.

TABLE 2

Effect of increasing amounts of mercuric chloride on the preservation of raw and heated milk for the determination of lactic acid

TREATMENT	AMOUNT OF $HgCl_2$ ADDED	LACTIC ACID VALUE AFTER HOLDING			PROTEIN NOT PRECIPITATED BY $Cu(OH)_2$ AFTER HOLDING			MICRO-ORGANISMS 12 weeks
		0	4 weeks	12 weeks	0	4 weeks	12 weeks	
	%	%	%	%	%	%	%	
Sweet skimmilk								
Raw	0.02	.000	.006	Mold	.13	.18	Mold	Present
Heated	0.02	.000	.001	Mold	.11	.17	Mold	Present
Raw	0.05	.000	.002	Mold	.11	.15	Mold	Present
Heated	0.05	.000	.002	.002	.11	.11	.15	Present
Raw	0.20	.000	.001	.003	.11	.13	.22	Absent
Heated	0.20	.000	.002	.001	.11	.11	.15	Absent
Raw	0.50	.000	.000	.003	.11	.12	.16	Absent
Heated	0.50	.000	.002	.001	.11	.10	.17	Absent
Raw	1.00	.000	.000	.003	.11	.13	.18	Absent
Heated	1.00	.000	.000	.000	.11	.11	.15	Absent
Partially soured skimmilk—Sample No. I								
Raw	0.02	.060	*.500	Mold	.14	.53	Mold	Present
Heated	0.02	.064	*.152	.611	.14	.12	.41	Present
Raw	0.05	.060	.069	Mold	.15	.33	.18	Present
Heated	0.05	.064	.068	.436	.12	.10	.16	Present
Raw	0.20	.060	.063	.074	.12	.18	.15	Absent
Heated	0.20	.064	.065	.070	.12	.10	.17	Absent
Raw	0.50	.060	.061	.073	.12	.11	.18	Absent
Heated	0.50	.064	.065	.081	.12	.10	.18	Absent
Raw	1.00	.060	.062	.068	.12	.11	.17	Absent
Heated	1.00	.064	.060	.070	.12	.11	.19	Absent
Partially soured skimmilk—Sample No. II								
Raw	0.02	.102	.103	Mold	.24	Mold	Mold	Present
Heated	0.02	.104	.111	Mold	.14	.14	Mold	Present
Raw	0.05	.102	.114	.136	.22	.64	1.25	Present
Heated	0.05	.104	.110	.116	.13	.14	.16	Present
Raw	0.20	.102	.107	.119	.12	.29	.65	Absent
Heated	0.20	.104	.107	.114	.12	.11	.17	Absent
Raw	0.50	.102	.104	.117	.12	.16	.28	Absent
Heated	0.50	.104	.107	.113	.12	.11	.17	Absent
Raw	1.00	.102	.102	.106	.12	.14	.25	Absent
Heated	1.00	.104	.106	.110	.12	.12	.16	Absent

* Ropy.

Pint samples were taken and two mercuric chloride tablets were added to each pint. Even if the tablets were pure mercuric chloride, the concentration of preservative was less than 0.2 per cent. The milk was not heated. Lactic acid determinations were made at the end of 2 and 9 days. The

results are presented in table 3. The endpoints in the acetaldehyde titration were not sharp in all cases. The odor of butyric acid could be detected in some of the samples and others contained large numbers of proteolytic bacteria. In this case an alteration in the sample occurred during a relatively short period of holding. This effect must have been due to enzymes.

TABLE 3

Lactic acid values of samples of raw milk taken from cans which were rejected at the receiving platform. Two mercuric chloride tablets per pint were added at once as a preservative

SAMPLE NUMBER	LACTIC ACID VALUE AFTER HOLDING THE PRESERVED SAMPLE FOR		
	2 days	9 days	Increase
	%	%	%
1	.001	.003	.002
2	.004	.010	.006
3	.004	.005	.001
4	.002	.009	.007
5	.002	.007	.005
6	.005	.011	.006
7	.009	.014	.005
8	.005	.009	.004
9	.010	.015	.005
10	.006	.011	.005
11	.004	.008	.004
12	.003	.012	.009
13	.001	.004	.003

The results presented in table 3 show that every one of the samples rejected because of an undesirable odor was either slightly sour or was abnormal in some other respect, as shown by the determination of lactic acid.

SUMMARY

Milk samples can be preserved satisfactorily for lactic acid determination up to one month when treated as follows:

Add not less than 0.5 per cent of mercuric chloride, then heat the sample to 80° C., for 5 minutes, cool and hold at 20° C., or lower, preferably in the dark.

The possibility of the use of small amounts of mercuric chloride as a preservative for studying the proteolytic action of milk is discussed.

REFERENCES

(1) CARPENTER, D. C. The influence of hydrogen ion concentration and of temperature on the hydrolytic scission of casein. Jour. Biol. Chem., v. 67, p. 647–658. 1926.

(2) TROY, H. C., AND SHARP, P. F. Quantitative determination of lactic acid in dairy products. Cornell Univ. Agr. Expt. Sta. Memoir 179. 1935.

(3) TROY, H. C., AND SHARP, P. F. Quantitative determination of lactic acid in butter. Cornell Univ. Agr. Expt. Sta. Memoir. In press.

VARIANTS OF STREPTOCOCCUS LACTIS WHICH DO NOT FERMENT LACTOSE

E. S. YAWGER, JR., AND J. M. SHERMAN
Cornell University, Ithaca, New York

The literature abounds with instances in which apparently pure bacterial strains show variation in the fermentation of sugars. In the fermentation reactions of any one species, there are frequently several test substances which may or may not be fermented. In many groups, however, the fermentation of some test substance is considered so characteristic that deviation from the usual reaction is used as a basis for a new species.

More than thirty years ago, Neisser (1) reported the occurrence of an organism belonging to the coli-aerogenes group which failed to ferment lactose. On lactose agar plates, however, this strain gave rise to secondary colonies which had the power of fermenting this sugar. Since that time many similar cases have been noted.

In the case of *Streptococcus lactis*, the fermentation of lactose and the souring of milk have been considered such basic characteristics that a strain which lacked these properties would not have been recognized as belonging to the group. However, Orla-Jensen (2) states that ". . . many strains prefer maltose to lactose. . . ." As *S. lactis* is usually isolated from milk or milk products, it seems logical to expect that non-lactose-fermenting strains, if present, would not grow so rapidly as the fermenting strains and hence would not be so readily isolated. *A priori*, there would appear to be no logical reason why non-lactose-fermenting variants of this species should not exist.

In a study of strains of *S. lactis* isolated from milk, four cultures were encountered which failed to curdle milk. Tests showed that they produced only a negligible amount of acid in milk; 0.03 to 0.04 per cent, calculated as lactic acid. At first it was thought that they were weakened strains, but an active fermentation in glucose broth, with a final limiting acidity of pH 4.0 to 4.2, indicated that this was not the case.

THE IDENTITY OF STREPTOCOCCUS LACTIS

Although *Streptococcus lactis* has long been known and extensively studied, its exact identity was not clarified until comparatively recent years. Among more observing bacteriologists, the complete reduction of litmus before curdling in milk cultures was long looked upon as an especially characteristic property of the organism (Hastings, 3). Esten (4), however, was misled in an important investigation by assuming that this typical action on litmus milk was not shared by any other streptococcus. Andrews and

Received for publication September 9, 1936.

Horder (5) had previously shown the strong reducing action of *S. fecalis* on neutral red, a substance more difficult to reduce, and MacCallum and Hastings (6), as early as 1899, had shown the same action in litmus milk cultures to be particularly characteristic of that gelatin-liquefying streptococcus which now goes under the name of *S. zymogenes*. It is now known that in addition to *S. lactis* and its relatives in the "lactic group," the ability to reduce litmus in milk prior to curdling is also a characteristic of the so-called "enterococcus group" consisting of *S. fecalis, S. zymogenes,* and *S. liquefaciens.*

The lactic acid streptococcus was first clearly defined by Sherman and Albus (7) who showed that, in addition to other characteristics, *S. lactis* has a minimum growth temperature below 10° C. and a maximum temperature for growth at about 43° C., and that these properties are correlated with the characteristic action in litmus milk. So far as present information extends, no streptococcus outside of the lactic group has this combination of characteristics. They also showed that *S. lactis* is not inhibited by dilute solutions of methylene blue, and gives the following fermentation reactions: the hexose sugars and lactose are fermented; raffinose, inulin, starch, and glycerol are not fermented; maltose, sucrose, mannitol, and salicin may or may not be fermented, though maltose usually is fermented while sucrose more often is not. Orla-Jensen (2) has shown that variable fermentation results are also obtained, within the species, on arabinose and xylose. It is also now known that typical cultures of *S. lactis* produce ammonia from peptone, but do not hydrolyze starch, as revealed by the starch agar plate technique (8, 9). Moreover, it has recently been shown that, in addition to their respective maximum temperatures of growth, *S. lactis* may be differentiated from *S. fecalis* on the basis of other tests (10).

There are, therefore, ample grounds on which to base a positive identification of *S. lactis,* even though the particular strain is a variant in some otherwise especially characteristic property.

THE CHARACTERISTICS OF THE VARIANT CULTURES

Four cultures which failed to ferment lactose were identified as *Streptococcus lactis* on the basis of the following combination of characteristics.

Growth took place at 10° C. and at 40° C., but no growth occurred at 45° C. In litmus milk cultures the litmus was completely reduced, but acid was not produced and the milk was not curdled. In litmus milk to which 2 per cent glucose was added, a reaction typical of *S. lactis* was obtained; the milk was rapidly acidulated and curdled, and the litmus was completely reduced prior to the curdling of the milk.

No growth occurred in broth containing 6 per cent sodium chloride; growth was not inhibited by medicinal methylene blue in a concentration of one part in 1,000 parts of skimmed milk; ammonia was produced in four

per cent peptone; carbon dioxide was not produced from glucose; starch was not hydrolyzed.

Glucose, fructose, maltose, and salicin were fermented; arabinose, lactose, raffinose, inulin, and glycerol were not fermented. The only variations obtained were with xylose, sucrose and mannitol, which are commonly known to represent variable characters within the species. Based on these three substances, there were two strains: one which failed to ferment xylose but fermented sucrose and mannitol, and another which fermented xylose but failed to ferment sucrose and mannitol.

In the light of present knowledge, it is permissible to consider these cultures definitely identified as members of the *S. lactis* group.

THE TAXONOMIC STATUS OF THE VARIANT

We recommend, for the present at least, that this variant be recognized only as such, within the species of *Streptococcus lactis*. Of course, if one were to follow the extreme differentiation, based on fermentation tests, advocated by Orla-Jensen and Hansen (11), this variant would have to be considered a new species. However, the absurdities to which such a policy would inevitably lead have previously been pointed out (9). In a species which shows so many other variations with the fermentation tests, there would appear to be no sound grounds for creating a new species on the basis of the non-fermentation of lactose. Strains which vary on this sugar are known in other species of bacteria, and, indeed, Rogers (12) has shown the soundness and logic of recognizing non-lactose-fermenting strains of *Bacterium aerogenes*.

When bacteriologists are finally driven to the realization that dependence must be put upon more basic characteristics, and a wider assortment of tests, the fermentation reactions will take their still important but secondary place in bacterial taxonomy.

That our views on this matter appear to be correct, so far as the present case is concerned, is indicated by the fact that after cultivation in milk for ten months, one strain is now able feebly to ferment lactose, and curdles milk after two weeks.

SUMMARY

Four cultures of *Streptococcus lactis* which did not ferment lactose were isolated from milk. These cultures were shown to agree with typical *S. lactis* strains in all other respects.

It is believed that these cultures represent naturally occurring variants of *S. lactis*, and this view appears to be borne out by the fact that one strain, after cultivation in milk for ten months, is gradually acquiring the ability to ferment lactose.

REFERENCES

(1) NEISSER, M. Cent. Bakt., I Abt. (Ref.), 36: 98. 1906.
(2) ORLA-JENSEN, S. The Lactic Acid Bacteria. Copenhagen. 1919.
(3) HASTINGS, E. G. *In* Marshall's Microbiology. Philadelphia. First Edition. 1911.
(4) ESTEN, W. M. Conn. (Storrs) Agr. Exp. Sta., Bul. 59. 1909.
(5) ANDREWS, F. W., AND HORDER, T. J. Lancet, 2: 708. 1906.
(6) MACCALLUM, W. G., AND HASTINGS, T. W. Jour. Exp. Med., 4: 521. 1899.
(7) SHERMAN, J. M., AND ALBUS, W. R. Jour. Bact., 3: 153. 1918.
(8) AYERS, S. H., JOHNSON, W. T., JR., AND MUDGE, C. S. Jour. Infect. Dis., 34: 29. 1924.
(9) STARK, PAULINE, AND SHERMAN, J. M. Jour. Bact., 30: 639. 1935.
(10) SHERMAN, J. M., AND STARK, PAULINE. JOURNAL OF DAIRY SCIENCE, 17: 525. 1934.
(11) ORLA-JENSEN, ANNA D., AND HANSEN, P. A. Zent. Bakt., II Abt., 86: 6. 1932.
(12) ROGERS, L. A. University of Wisconsin Studies in Science, 2: 76. 1921.

A FURTHER STUDY OF THE FACTOR IN SOYBEANS AFFECTING THE VITAMIN A VALUE OF BUTTER[1]

S. M. HAUGE, J. W. WILBUR AND J. H. HILTON
Research Chemical Laboratory and Department of Dairy Husbandry, Purdue University Agricultural Experiment Station, Lafayette, Indiana

In previous publications (1, 2, 3), it had been shown that whenever soybeans were introduced into rations of dairy cows, either through soybean hay or as part of the grain rations, the soybeans had a suppressing action on the formation of vitamin A in butter. The possibility that the factor responsible for the suppression of the vitamin A value in milk fat might be the same as that responsible for the repressed growth of rats and hogs (4, 5, 6) was eliminated, when it was found that roasting the soybeans, a treatment which improves the nutritive value of soybeans for growth (4), did not modify the factor responsible for the lowered vitamin A value of butter (2).

Furthermore, the stability of this factor to the heat of the roasting process (2) and to the temperatures used in the drying of soybean hay with the mechanical drier (3), indicates that it is not thermo-labile.

Since this factor is found in the beans, experiments were conducted for the purpose of determining what component part or parts of the bean are responsible for this action. There existed the possibility that on extraction of the oil from the beans, this factor might be found to be associated entirely with the oil, or that it might be retained by the meal. If the factor was not removed from the bean by the extraction of the oil, there still existed the possibility that it might be eliminated by further treatment with various solvents.

EXPERIMENTAL

The soybeans used in these experiments were secured from the same source. Some were treated in a commercial soybean plant and others subjected to various treatments in the chemical laboratory. The following products were prepared and used in the feeding trials:

1. Soybeans. These were raw and untreated.
2. Soybean Oil Meal (Expeller Process). This was prepared in a commercial soybean plant by the regular expeller process.
3. Soybean Oil (Expeller Process). This was the oil from the above meal.

Received for publication September 8, 1936.

[1] Published with the approval of the Director of the Purdue University Agricultural Experiment Station.

4. Soybean Oil Meal (Solvent Method). This product was prepared by prolonged extraction of ground soybeans, first with ethyl ether and then with ethyl alcohol in a Lloyd's Extraction Apparatus.

5. Soybean Oil (Solvent Method). This oil was secured during the first step of the extraction process as described under No. 4, when the beans were extracted with ethyl ether.

6. Alcoholic Extract. This product was secured during the second step of the extraction process as described under No. 4, when ethyl alcohol was used as the solvent.

In addition to these soybean products, linseed oil meal and linseed oil were used in some of the rations of the feeding trials.

Four Guernsey cows divided into two groups of two cows each were used in the feeding experiments. All cows were in the early stages of lactation at the beginning of the feeding trials and continued throughout the tests in a fairly uniform daily milk production. All cows received alfalfa hay, corn silage and a basal grain mixture of 400 pounds ground white corn and 200 pounds ground oats. The alfalfa hay furnished the principal source of vitamin A in the ration. The hay was of excellent quality and was fed in uniform amounts throughout the trials. The corn and oats mixture was modified by the addition of the various ingredients studied during the various feeding periods. Each feeding period was of 21 days duration. Group A received in addition to the basal ration the following ingredients added to the grain mixture in successive feeding periods: A-1, 100 pounds linseed oil meal; A-2, 100 pounds raw soybeans; A-3, 100 pounds soybean oil meal (expeller) and A-4, 100 pounds linseed oil meal plus 5 per cent linseed oil.

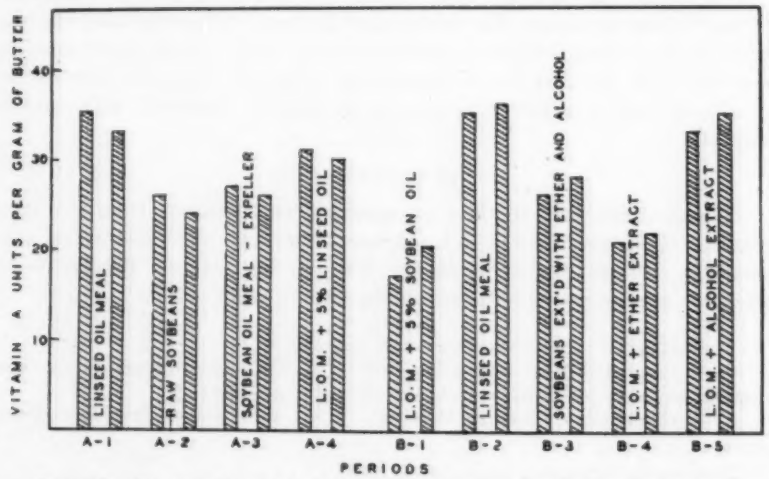


CHART I. Showing the effect of the vitamin A suppressing factor found in soybeans and its products upon the vitamin A value of butter.

The corn and oats mixture of Group B was modified as follows in successive feeding periods: B-1, 100 pounds linseed oil meal plus 5 per cent soybean oil; B-2, 100 pounds linseed oil meal; B-3, 100 pounds soybean oil meal (solvent process); B-4, 100 pounds linseed oil meal plus soybean oil from ether extraction from 100 pounds soybeans and B-5, 100 pounds linseed oil meal plus alcoholic extract from 100 pounds fat free soybean oil meal.

Representative samples of milk were collected from each cow at the end of each 21 day feeding period, the cream separated and churned into butter. Each butter sample was then subjected to biological assays for vitamin A, using the technique previously described (1).

The results of these assays are given in Chart I and table 1, the values being expressed in Sherman and Munsell (7) vitamin A units.

TABLE 1

Showing the rations fed the cows during each period and the vitamin A activity of the butterfat produced by cows fed the various supplements

PERIOD NO.	ROUGHAGE		GRAIN RATION			COW NO.	VITAMIN A UNITS PER GRAM (BUTTER)
	Hay	Silage	White Corn	Oats	Supplements		
A1	Alfalfa	Corn	400 lbs.	200 lbs.	100 lbs. linseed oil meal (check)	514 438	35 33
A2	"	"	"	"	100 lbs. raw soybeans	514 438	26 24
A3	"	"	"	"	100 lbs. soybean oil meal (expeller)	514 438	27 26
A4	"	"	"	"	100 lbs. linseed oil meal plus 5 per cent linseed oil	514 438	31 30
B1	"	"	"	"	100 lbs. linseed oil meal plus 5 per cent soybean oil	513 439	17 20
B2	"	"	"	"	100 lbs. linseed oil meal (check)	513 439	35 36
B3	"	"	"	"	100 lbs. soybeans (extracted with ether and alcohol)	513 439	26 28
B4	"	"	"	"	100 lbs. linseed oil meal plus soybean oil from ether extraction from 100 lbs. soybeans	513 439	21 22
B5	"	"	"	"	100 lbs. linseed oil meal plus alcoholic extract from 100 lbs. soybeans	513 439	33 35

DISCUSSION

In these experiments, alfalfa hay was the chief source of vitamin A in the ration fed the cows. Since the amount of alfalfa fed each cow daily was

so regulated that the potential vitamin A values of the rations were the same in each lot and throughout the successive feeding periods, any difference in the vitamin A values of the butter could only be attributed to the effects of the various supplements in the grain mixtures.

From the results presented in Chart I it can be seen that butters made from the milk of cows fed the check ration (linseed oil meal) possessed high vitamin A values. The lower vitamin A value of the butter secured when raw soybeans were substituted for linseed oil meal in the grain ration of the cows shows the effect of the factor in soybeans responsible for the suppression of vitamin A activity of butter.

In studying the different fractions prepared from soybeans, it is interesting to note that on the removal of the oil either by the expeller process or by chemical solvents much of this factor followed the oil. When five per cent of the oil was added to the check ration, there was a marked suppression of the vitamin A value of the butter.

This suppressing action of the soybean oil might appear to be due to the oil itself. However, when the butters secured by feeding five per cent soybean oil are compared with those secured from feeding five per cent linseed oil, it becomes apparent that this effect is not produced by the presence of oil in the ration but rather by something in the soybean oil which apparently is not present in linseed oil.

Although a good portion of this factor is present in the soybean oil, the soybean oil meal (expeller process) contains significant amounts. Even after the complete removal of the oil by exhaustive extraction with ethyl ether followed by similar extraction with ethyl alcohol, this factor still remains in the soybean oil meal. This further indicates that the suppressing action is not due to the oil itself but to some other factor distributed in both the oil and the soybean oil meal.

When the alcohol extract, obtained by extraction with ethyl alcohol of the soybean oil meal which had been previously extracted with ether to remove the oil, was included in the rations of the cows, butter was obtained which possessed high vitamin A activity. Since the soybean oil meal still contained the suppressing factor, it becomes evident that this factor is not soluble in alcohol, or at least not extractable by alcohol after the removal of the oil.

From these studies on the interfering action of soybeans and soybean products on the formation of vitamin A in butter, it is apparent that there is a definite factor responsible for this action, which is stable to heat, insoluble in alcohol and found in the soybean seed, soybean oil and soybean oil meal.

SUMMARY

1. Further studies have been made of the vitamin A suppressing factor in soybeans which interferes with the transference of the vitamin A activity of the feed to the butterfat secreted by dairy cows.

2. This factor was found to be distributed in both the soybean oil and soybean oil meal secured by either the expeller process or by chemical solvents.

3. The suppressing action is not due to the presence of oil in the ration but to some factor in soybean oil in the bean.

4. Prolonged extraction of soybeans first with ethyl ether and then with ethyl alcohol failed to completely remove this factor.

5. The inability of alcohol to extract the factor from fat free soybean oil meal indicates that it is not soluble in ethyl alcohol.

REFERENCES

(1) Hilton, J. H., Hauge, S. M. and Wilbur, J. W. Maintaining the Vitamin A Value of Butter through Winter Feeding Conditions. Journal of Dairy Science 16: 355–361. 1933.

(2) Wilbur, J. W., Hilton, J. H. and Hauge, S. M. The Effect of Soybeans in the Rations of Dairy Cows upon the Vitamin A Value of Butter. Journal of Dairy Science 18: 661–665. 1935.

(3) Hilton, J. H., Hauge, S. M. and Wilbur, J. W. The Vitamin A Activity of Butter Produced by Cows fed Alfalfa Hay and Soybean Hay Cut in Different Stages of Maturity. Journal of Dairy Science 18: 795–800. 1935.

(4) Shrewsbury, C. L., Vestal, C. M. and Hauge, S. M. The Effect of Yeast and Casein Supplements to Corn and Soybean Rations when fed to Rats and Swine. Journal Agr. Research 44: 277–281. 1932.

(5) Osborne, S. B., and Mendel, L. B. The Use of Soybeans as Food. Journ. Biol. Chem. 32: 369–387. 1917.

(6) Robinson, W. L. Soybeans and Soybean meal for pigs. Ohio Agr. Exp. Sta. Bul. 452. 1930.

(7) Sherman, H. C. and Munsell, H. E. The Quantitative Determination of Vitamin A. Jour. Amer. Chem. Soc. 47: 1639–1646. 1925.

A SIMPLE PLANT METHOD OF ESTIMATING THE ALKALINE CONSTITUENTS OF WASHING POWDERS AND WASHING SOLUTIONS CONTAINING MIXED ALKALIES

LAWRENCE L. LITTLE
Meadow Gold Milk Plant, Oklahoma City, Oklahoma

With the new developments in cleaning in the dairy industry and the great number of new cleaning powders on the market, each making astounding claims for their product, the dairy plant manager is greatly in need of a simple, rapid plant test by which he can determine the approximate ingredients of the various cleaners in order to evaluate their worth. Many plant operators use a mixture of two or more alkalies in the soaker compartment of their bottle washing machine. Some alkalies possess the ability of maintaining their strength in solution longer than others. Therefore in order to maintain a constant balance between the different alkalies it becomes necessary for the plant operator to be able to determine the amount of each alkali present at frequent intervals.

The most satisfactory method of determining the ingredients in a solution of mixed alkalies consists in titrating a sample of the alkali with standard acid to the phenolphthalein end point, taking the reading and continuing the titration to the methyl orange end point. The first titration minus the second is considered to represent the caustic soda portion. The methyl orange titration represents one half of the soda ash portion. When silicates or phosphates are present, a saturated solution of barium chloride is added and the phenolphthalein titration is considered as caustic soda, while the methyl orange titration is calculated as soda ash. This method will give correct results only when the solution contains only caustic soda and soda ash. When silicates are present they will largely be included in the caustic soda portion, while phosphates will be largely included in the soda ash portion. Myers (2) recommends the use of an additional indicator, trinitrobenzene, in the analysis of mixed alkalies, but we were unable to use this method, or any of the many other modifications, with any satisfaction when the alkali included either silicates or phosphates in the presence of soda ash and caustic soda. The alkalies most commonly used in dairy washing powders are caustic soda, soda ash, sodium meta silicate, and trisodium phosphate. Practically all dairy washing powders are made up of these alkalies, either singly or by combining them in various proportions. A detailed study was made of the four alkalies in view of finding a selective reaction or method of treatment which could be used to analyze each ingredient when in the presence of one or more of the others.

Figure 1 shows the approximate pH of solutions of each of the four alkalies from 0.1 to 4.0% concentration. A study of this chart will reveal

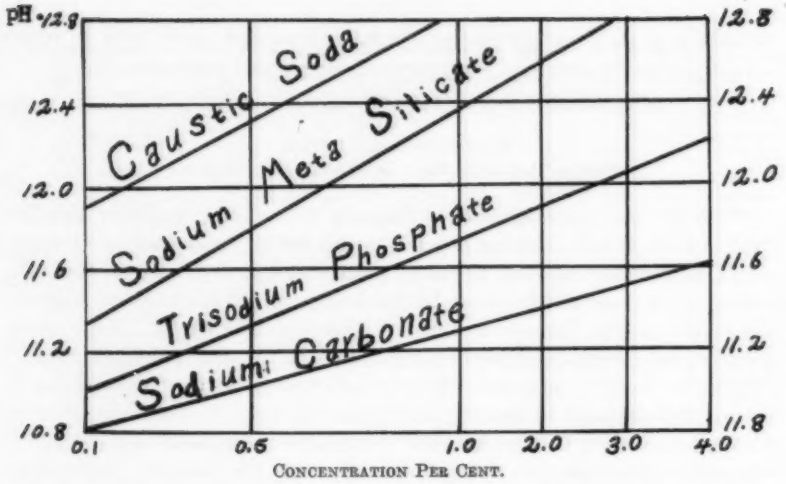


FIG. 1. THE APPROXIMATE pH OF SOLUTIONS OF CAUSTIC SODA, SODA ASH, TRISODIUM PHOSPHATE, AND SODIUM META SILICATE OF VARYING CONCENTRATIONS.*

that they can not be distinguished by the use of different indicators alone. The results of a detailed study of the alkalies is presented in table 1. The caustic soda is 100% active at the phenolphthalein end point, while sodium meta silicate is 93.5% active and soda ash is 50% active. It is usually considered that trisodium phosphate is one third active at the phenolphthalein end point, one third active at the methyl orange end point and one third inert at the methyl orange end point, however in this study the average of all the samples analyzed was 42.12% active at the phenolphthalein end point and all of the samples studied were close to this average, falling within the range of from 40 to 45%. The average for all the trisodium phosphate samples analyzed was 35.70% active from the phenolphthalein to the methyl orange end point. We considered this to be one third of the trisodium phosphate, and for plant analysis the error in considering it thus will be so small that it can be ignored. With the exception of trisodium phosphate, all the other alkalies were completely active at the methyl orange end point. The average for the trisodium phosphate samples being 22.18% inert at the methyl orange end point.

The first procedure was to find a method for determining caustic soda which would exclude all the other alkalies. An attempt was made to precipitate the sample with barium chloride and filter out all but the caustic soda portion. This will remove all the alkalies but a small portion of the sodium meta silicate, but this could not be completely removed by filtering

* From material presented by the Philadelphia Quartz Co.

through the finest filter paper. Sodium hydroxide is soluble in hot alcohol while all the others are insoluble, but when a solution of the alkalies is dissolved in alcohol, a considerable part of the sodium meta silicate will be soluble. By precipitating the solution with barium chloride and adding alcohol and boiling for a few minutes and titrating while hot, the caustic soda is from 98 to 100% soluble while all the other alkalies are totally insoluble, thereby furnishing a method of determining sodium hydroxide when in solution with the three other alkalies. When a saturated solution of barium chloride is added to the alkali and the solution boiled for a few minutes, cooled and titrated, the sodium hydroxide was 100% soluble, sodium meta silicate was 93.5% soluble, the soda ash totally insoluble and the trisodium phosphate was only approximately 1.5% soluble. This titration will include, for all practical purposes, only the sodium hydroxide and sodium meta silicate. Hence by subtracting the sodium hydroxide portion from the portion representing the sodium hydroxide and sodium meta silicate, the silicate portion can be determined.

Trisodium phosphate and soda ash are the only alkalies which form a reversible reaction, (3) therefore by titrating the solution to the methyl orange end point and boiling the solution to break down the H_2CO_3 and expelling the CO_2, and titrating back to the phenolphthalein end point a differential reaction for phosphates is established. Approximately one third of trisodium phosphate is active from the phenolphthalein to the methyl orange end point. The solution is titrated back with standard alkali until the solution is alkaline to methyl orange, and the reading taken and the titra-

TABLE 1

Some properties of the alkalies

DISTINGUISHING PROPERTIES OF THE ALKALIES	ALKALIES			
	Per cent of total alkaline ingredients			
	NaOH	Na_2CO_3	Na_3PO_4	Na_2SiO_3
Active at phenolphthalein end point	100.00	50.00	42.12	93.50
Active from phenolphthalein to methyl orange end point	.....	50.00	35.70	6.50
Inert portion of alkali	.....	.....	22.18	.....
Solubility in hot alcohol after precipitating with barium chloride. Phenolphthalein	98.00	.....	.....	.....
Solubility at phenolphthalein end point after ppt. with barium chloride and boiling	100.00	.....	1.59	93.50
Solubility from phth. to methyl orange end pt. after ppt. with barium chloride and boiling	.....	97.70	61.97	2.70
Reversible reaction after boiling to expel CO_2	.....	.....	99.75	.....

tion continued to the phenolphthalein end point. The portion from the methyl orange end point to the phenolphthalein end point represents one third of the trisodium phosphate. This completes the determination of three of the four constituents, and the fourth one, soda ash, may be determined by the difference between the total acid required to bring the solution to the methyl orange end point and the amount representing the total of the other three alkalies.

Table 2 gives the percentage composition of each of the alkalies for each of the steps in the analysis, and the total composition is given in table 3.

TABLE 2

Percentage of active ingredients in the alkalies as determined by each step in the proposed methods

	Na_3PO_4-$12H_2O$ TECH.	NaOH (95% NaOH + 5% Na_2CO_3) COMM.	Na_2CO_3-H_2O C. P.	Na_2SiO_3-$5H_2O$ COMM.
Active Na_2O (phenolphthalein end point)	11.22	77.58	25.79	28.40
Inactive Na_2O (methyl orange end point)	9.06	1.48	25.79	1.97
Total Na_2O	20.28	79.06	51.58	30.37
$BaCl_2$ + alcohol and boil	.....	96.25	.....	.....
$BaCl_2$ and boil	1.59	96.25	.....	95.50
Back titration after boiling	99.75	.....	.....	.....
Phenolphthalein end point	135.40	98.00	101.50	95.50
Methyl orange end point	106.90	1.75	101.50	6.50
Total (phenolphthalein and methyl orange)	121.10	99.75	101.50	102.00

TABLE 3

Percentage composition of the alkalies

CONSTITUENTS	COMMERCIAL CAUSTIC SODA 95% NaOH 5% Na_2CO_3	C. P. SODA ASH Na_2CO_3-H_2O	COMMERCIAL SODIUM META SILICATE Na_2SiO_3-$5H_2O$	TECHNICAL TRISODIUM PHOSPHATE Na_3PO_4-$12H_2O$
NaOH	95.00	.....	.....	.....
Na_2CO_3	4.63	86.72	.....	.....
Na_2CO_3-H_2O	.....	101.50	.....	.....
Na_3PO_4	.....	.....	.....	43.05
Na_3PO_4-$12H_2O$	.....	.....	.....	99.75
Na_2SiO_3	.....	.....	58.67	.....
Na_2SiO_3-$5H_2O$	.....	.....	102.00	.....
Moisture and inert material by difference	0.37	13.28	41.33	56.95

A washing powder was made up of equal parts by weight of each of the four alkalies and analyzed according to the proposed method, the results being shown in table 4. The analysis checked uniformly closely with the actual composition, the greatest variation being -2.47% for Na_2SiO_3-$5H_2O$.

TABLE 4

Analysis of a washing powder made up of equal parts by weight of caustic soda, soda ash, trisodium phosphate and, sodium meta silicate

CONSTITUENTS	ACTUAL COMPOSITION	ANALYSIS	ERROR
NaOH	23.75	23.75	———
Na_2CO_3	22.93	22.51	− .42
Na_2CO_3-H_2O	26.25	26.35	+ .10
Na_3PO_4	10.76	11.28	+ .52
Na_3Po_4-$12H_2O$	25.00	26.13	+1.13
Na_2SiO_3	14.68	12.96	+1.72
Na_2SiO_3-$5H_2O$	25.00	22.53	−2.47
Total alkaline ingredients	72.12	70.50	−1.62
Active Na_2O	35.75	35.70	− .05
Inactive Na_2O	9.58	8.73	− .85
Total Na_2O	45.33	44.43	− .90
Moisture and inert material by difference	27.78	29.50	+1.72

A 4.0% solution of the washing powder was analyzed and the results given in table 5. The analysis was much closer to the actual composition in the washing solution than for the washing powder, the reason of course being due to the multiplication of the error in calculating the results for the washing powders.

As shown in table 5, the maximum error was −.10% calculated as Na_2SiO_3-$5H_2O$ and only −.07% calculated as Na_2SiO_3; therefore for a plant test as a check on the washing solutions, this method would give very satisfactory results, the error being so slight that it would be insignificant.

PROPOSED METHOD OF ANALYSIS

The following outline is the proposed method for analyzing a washing powder containing any or all of the four cleaning alkalies, caustic soda, soda ash, trisodium phosphate, and sodium meta silicate.

1. Weigh out ten grams of the washing powder and transfer to a 250 cc. volumetric flask and fill to the mark with distilled water.

2. Shake thoroughly until all the alkali is dissolved and the solution becomes homogeneous throughout.

3. Pipette 10 cc. of the solution into a 200 cc. pyrex Erlenmeyer flask, and titrate to the phenolphthalein end point with N/4 sulfuric acid. Record the cc. of acid required as (1). Now add methyl orange indicator and titrate to the end point. Record this titration as (2). Heat the solution gently over a hot plate or on an asbestos buffer over a Bunsen burner, bringing to a gentle boil, and boiling slowly for 15 minutes. Cool to room temperature and titrate back to the alkaline side of the methyl orange end point with N/4 NaOH. Record this titration as (3). Continue the titration to the very faintest pink phenolphthalein end point. Record this titration as (4).

TABLE 5

Analysis of a 4.0% solution of a washing powder made up of equal parts by weight of caustic soda, soda ash, trisodium phosphate and sodium meta silicate

CONSTITUENTS	ACTUAL COMPOSITION	ANALYSIS	ERROR
Na_2CO_3	0.92	0.90	− .02
NaOH	0.95	0.95	.....
Na_2CO_3-H_2O	1.05	1.05	.....
Na_3PO_4	0.43	0.45	+ .02
Na_3PO_4-$12H_2O$	1.00	1.05	+ .05
Na_2SiO_3	0.59	0.52	− .07
Na_2SiO_3-$5H_2O$	1.00	0.90	− .10
Total alkaline ingredients	2.89	2.82	− .07
Active Na_2O	1.43	1.43	.....
Inactive Na_2O	0.38	0.35	− .03
Total Na_2O	1.81	1.78	− .03

4. Pipette another 10 cc. sample into a 200 cc. pyrex Erlenmeyer flask. Add 10 cc. of a saturated solution of barium chloride and boil vigorously for five minutes. Cool to room temperature and titrate to the phenolphthalein end point with N/4 sulfuric acid. Record this titration as (5).

5. Pipette another 10 cc. sample into a 200 cc. pyrex Erlenmeyer flask. Add about one gram of pure barium chloride crystals and shake vigorously. Allow to stand for 10–15 minutes and add 50 cc. of 95% neutral ethyl alcohol (neutral denatured alcohol will suffice). Heat very slowly on a hot plate or on an asbestos buffer over a bunsen burner to boiling, being very careful not to ignite the fumes from the alcohol. Shake vigorously throughout the heating process to prevent bumping. Boil gently for ten minutes, and titrate while hot to the phenolphthalein end point with N/4 sulfuric acid. Record this titration as (6).

Consider (1) + (2) as A. Twice (3) + (4) as B, and (5) as C.

Calculation results:

$(1) \times 1.94 = \%$ active Na_2O in the sample.
$(2) \times 1.94 = \%$ reserve Na_2O in the sample.
$(A) \times 1.94 = \%$ total Na_2O in the sample.
$(4) \times 23.75 = \%$ $Na_3PO_4 - 12\,H_2O$ in the sample.
$(4) \times 10.25 = \%$ Na_3PO_4 (anhydrous) in the sample.
$(6) \times 2.5 = \%$ NaOH in the sample.
$(5-6) \times 6.63 = \%$ $Na_2SiO_3 - 5\,H_2O$ in the sample.
$(5-6) \times 3.81 = \%$ Na_2SiO_3 (anhydrous) in the sample.
$A-(B+C) \times 3.31 = \%$ Na_2CO_3 in the sample.
$A-(B+C) \times 3.88 = \%$ $Na_2CO_3 - H_2O$ in the sample.

To analyze a sample of washing solution, cool to room temperature and pipette 9.6 cc. (10 grams) of the well mixed solution for a sample instead

of using 10 cc. as in analyzing a washing powder. Follow the same procedure used for washing powder but begin with step 3.

Calculation of results:

(1) × .0775 = % active Na_2O in the solution.
(2) × .0775 = % reserve Na_2O in the solution.
(A) × .0775 = % total Na_2O in the solution.
(4) × .95 = % $Na_3PO_4 - 12\ H_2O$ in the solution.
(4) × .41 = % Na_3PO_4 (anhydrous) in the solution.
(6) × .10 = % NaOH in the solution.
(5 − 6) × .153 = % Na_2SiO_3 (anhydrous) in the solution.
(5 − 6) × .265 = % $Na_2SiO_3 - 5\ H_2O$ in the solution.
A − (B + C) × .133 = % Na_2CO_3 in the solution.
A − (B + C) × .155 = % $Na_2CO_3 - H_2O$ in the solution.

REFERENCES

(1) H. F. Zoller. Certain fundamental factors involved in cleaning dairy equipment. Proc. Intern. Assoc. Milk Dealers, Lab. section, 21: 35–52. 1928.
(2) R. P. Myers. Germicidal properties of alkaline washing solutions, with special references to the influence of hydroxyl-ion concentration buffer index, and osmotic pressure. Jour. Agr. Research, 38: 521–63. 1929.
(3) Scott. Standard methods of chemical analysis. Vol. 2, pp. 1521.
(4) Barnum, Lucas, and Hartsuch. The use of cleaners in the dairy plant. Spec. Bul. No. 262. Agri. Exp. Sta. Michigan State College. 1935.
(5) Phillips, A. W., M. J. Mack, and J. H. Frandsen. Mass. Agri. Exp. Station Tech. Bul. 13. 1928.
(6) A. H. Johnson. Washing and sterilizing solutions. Methods for the determination of the concentration of washing solutions. Proc. Intern. Assoc. Milk Dealers. Lab. Section, 23: 44–8. 1930.

FERMENTATIVE VARIABILITY AMONG SUBSTRAINS OF STREPTOCOCCUS CREMORIS AND STREPTOCOCCUS LACTIS OBTAINED FROM PURE CULTURES

J. M. SHERMAN AND R. V. HUSSONG
Cornell University, Ithaca, New York

Sherman and Wing (1) have shown that if pure cultures of *Bacterium coli* and *Bacterium aerogenes* are plated out by the poured agar plate method, some of the substrains isolated vary in their fermentative capacities from the parent strains. We undertook similar work with *Streptococcus cremoris* and *S. lactis* because of their close relationship to each other, and also because the fermentation tests have been advocated as means of differentiating these two species. Again, it was thought this study might throw light upon the soundness of the suggestion that many species be recognized among the "lactic" streptococci, on the basis of slight differences in their fermentation reactions.

EXPERIMENTAL

For this work we used a culture of *S. cremoris* which came originally from the laboratory of Dr. Orla-Jensen, and was obtained by us from Dr. G. J. Hucker. This culture fermented neither maltose nor sucrose. Our culture of *S. lactis* was isolated in this laboratory and had been in stock for a number of years. It fermented maltose but not sucrose.

Litmus milk cultures of various ages, which had been incubated at temperatures ranging from that of the laboratory up to 35° C., were plated out by the poured agar plate method. After incubation, isolations were made from well separated colonies. The substrains so obtained were tested for their abilities to ferment maltose and sucrose. A summary of the results obtained is given in Table 1.

TABLE 1

Substrain variation on fermentation tests

PARENT CULTURE	TOTAL SUBSTRAINS	NUMBER MALTOSE − SUCROSE −	NUMBER MALTOSE + SUCROSE −	NUMBER MALTOSE − SUCROSE +	NUMBER MALTOSE + SUCROSE +
S. cremoris	458	*217	229	11	1
S. lactis	757	0	*756	0	1

* Type of parent culture.

It is readily seen that the culture of *S. cremoris* gave rise to numerous substrains which varied from the parent culture. The culture of *S. lactis*, on the other hand, was more stable in this respect; only one substrain which varied from the parent type was obtained. It is of considerable interest to

note that in the case of *S. cremoris,* more substrains were obtained which were (maltose +, sucrose –) than were obtained of those which resembled the parent culture (maltose –, sucrose –). The next experiment throws additional light on this phenomenon.

Substrains, from the original culture of *S. cremoris,* which were (maltose –, sucrose –), (maltose +, sucrose –) and (maltose +, sucrose +) were plated and about 100 colonies isolated from each. Unfortunately, we had not retained a substrain which was (maltose –, sucrose +) from our earlier experiments, and this type was not represented in these tests. The results of this experiment are given in Table 2.

TABLE 2

Fermentative variation among strains isolated from different substrain types from the original culture of Streptococcus cremoris

SUBSTRAIN	PER CENT MALTOSE – SUCROSE –	PER CENT MALTOSE + SUCROSE –	PER CENT MALTOSE – SUCROSE +	PER CENT MALTOSE + SUCROSE +
Maltose –, Sucrose –	14	86	0	0
Maltose +, Sucrose –	5	95	0	0
Maltose +, Sucrose + ...	27	*73	0	*0

* Many of these cultures showed a very slight acidity in sucrose broth, indicating a feeble fermentation.

These data again show the strong tendency among the offspring of the parent culture to ferment maltose; and in addition the tendency to lose the power of fermenting sucrose, among the descendants of substrains which had that power.

The stock culture of *S. cremoris* used in these experiments, which was maintained in milk culture, was tested after the completion of the work and was still (maltose –, sucrose –). On this same point, in connection with our work on the colon bacteria (1), it was said: "It would seem probable that individual cell variants commonly occur in stock cultures just as they do when isolated in agar. It is an exceedingly nice question, which we shall not now attempt to answer, why these variants do not ordinarily make their presence evident in mass cultures. It is believed, however, that the data herein reported throw light on those relatively rare but authentic instances in which stock cultures have changed in their fermentative capacities."

DISCUSSION

This work confirms that of Sherman and Wing with colon bacilli, and presents some problems of interest and importance to students of the lactic acid streptococci. From the standpoint of classification, serious questions are raised concerning the value of the fermentation tests in the identification of the species, or varieties, which are contained in the general group.

The *S. lactis* group, using the term in a broad sense, is known to contain organisms which ferment, and others which do not ferment, arabinose, xylose, maltose, sucrose, mannitol and salicin. Orla-Jensen and Hansen (2) have reported a type which ferments raffinose, and Yawger and Sherman (3) have recently found a few strains which do not ferment lactose. Hence, there are eight test substances on which fermentative variability is known to occur within the group. Stark and Sherman (4) have shown that with arabinose, xylose, sucrose, and mannitol, all of the theoretically possible 16 combinations, with four variable characters, are to be found among the cultures of *S. lactis* described in the literature—rather strong evidence that these variations are random ones and not of taxonomic significance. If the number were increased to include the eight test substances known to give variable results, the possible number of combinations becomes 256. Were species to be recognized on such a basis, the mere selection of appropriate names for the new "species," shortly coming to light, would tax the ingenuity of a scholar of the classics.

This is not meant to imply that *S. cremoris* is not a species distinct from *S. lactis;* only that it is doubtful if a differentiation can be founded on the fermentation tests. As a matter of fact, we are inclined strongly to the belief that *S. cremoris* is a distinct species, and work now in progress in this laboratory leads us to hope that it will be possible, for the first time, to define this organism clearly.

SUMMARY

From a pure culture of *Streptococcus cremoris* which fermented neither maltose nor sucrose (maltose –, sucrose –), 458 substrains were isolated by the poured agar plate method. Of these, 217 were (maltose –, sucrose –), 229 were (maltose +, sucrose –), 11 were (maltose –, sucrose +), and one was (maltose +, sucrose +). A culture of *S. lactis* (maltose +, sucrose –) was more stable, yielding 756 substrains which were (maltose +, sucrose –), and one which was (maltose +, sucrose +).

REFERENCES

(1) Sherman, J. M., and Wing, Helen U. Jour. Bact. (In press).
(2) Orla-Jensen, Anna D., and Hansen, P. A. Zent. Bakt., II Abt. 86: 6. 1932.
(3) Yawger, E. S., Jr., and Sherman, J. M. Journal of Dairy Science. (In press).
(4) Stark, Pauline, and Sherman, J. M. Jour. Bact. 30: 639. 1935.

THE DETECTION AND SIGNIFICANCE OF ESCHERICHIA–AEROBACTER IN MILK[1, 2]

III. CORRELATION OF TOTAL BACTERIAL COUNT AND PRESENCE OF THE COLI–AEROGENES GROUP

M. T. BARTRAM AND L. A. BLACK
Department of Bacteriology, University of Maryland, College Park

INTRODUCTION

The coli-aerogenes group has long been used as an indicator of the sanitary quality of water. Studies of the prevalence of this group in milk and milk products have not been sufficiently extensive to permit definite conclusions. Many investigators believe that the total bacterial count is insufficient to show completely the sanitary quality of the milk and particularly of pasteurized milk, careless handling or insufficient heating may result in significant contamination and yet the total count may not be markedly increased. In such cases, the examination for members of the coli-aerogenes group may be of importance. However, it must be remembered that Beavens (1), and Minkin and Burgwald (2) showed that strains resistant to pasteurization may occur in milk. Stark and Patterson (3) tested 505 cultures from water, milk and feces and found all to be destroyed by 145° F. for 30 minutes.

Since recent reports have not cited much of the outstanding work on the significance of the colon group in milk, a portion of the literature on this subject will be briefly reviewed. Work, prior to 1925, on the isolation and significance of the colon bacilli in milk was done by Ayres, Cook and Clemmer (4); Hunter (5); and Finkelstein (6). The first mentioned investigators concluded that the colon group could be used to a limited extent in determining the efficiency of the cooling of the milk. Hunter observed a close correlation between total count and the number of colon organisms. Finkelstein found that the coli-aerogenes count would be under 100 per cc. in carefully handled raw milk. In pasteurized milk, he found that they were reduced to an average of 42 per cc. and in some cases none were found. However, the methods of isolating colon bacilli have been changed and the care used in the production of milk has increased. Later, Swenarton (7), using gentian violet lactose peptone bile medium, found that a high count of *B. coli* correlated with plants showing improper heating or cooling of milk.

Received for publication September 14, 1936.

[1] Approved for publication by the director of the Maryland Agricultural Experiment Station.

[2] A portion of a thesis submitted to the Graduate School of the University of Maryland by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Kline (8) found that there was little correlation between colon and total count in low count milk, and that in high count milk the correlation was insignificant. The same general consideration held true in pasteurized milk but in certified milk there was a definitely lower percentage of samples with a count of under 10,000 per cc. showing colon. Malcolm (9) found a definite correlation between the average bacterial count and the coli-form-positive samples. Munchberg (10) observed a close relationship between the coli titre and bacterial or chemical tests for contamination.

Sherman and Wing (11) concluded that the colon test is of no special value as an index to the sanitary condition of the usual grade of raw milk, but in milk of under 10,000 total count, the colon test may act as a supplementary index of quality. In such milk these organisms should number less than 100 per cc. In certified milk they believed the recommendation of the American Association of Medical Milk Commission of less than 10 colon organisms per cc. to be satisfactory.

Barkworth (12), in a statistical examination of the interrelationship of the plate count, coliform content, and keeping quality, found that the variabilities were too great to permit forecasting one term from the other, but that an increase of coliform organisms reduced the keeping quality of the milk to the same extent as a seven-fold increase in plate count. Moldavan (13) stated that "it is generally agreed that in most cases, positive results from samples of pasteurized milk are due to non-fecal organisms, the presence of which indicates faulty sterilization and improper pasteurization rather than fecal contamination."

McCrady and Langevin (14) found the coli-aerogenes organisms were practically absent from one cc. quantities at the pasteurizing vat but that contamination often occurred in the cooling or bottling process, although it appeared that properly pasteurized milk should not contain these organisms in one cc. portions in more than 10 to 20 per cent of tests.

Slack and Maddeford (16), in examining 25 samples of raw milk sold to pasteurizing plants, found 4 per cent to contain no colon organisms in 10 cc. of milk, 12 per cent to be positive only in 10 cc., 12 per cent positive in 1 cc., 20 per cent positive in 0.1 cc., and 52 per cent positive in amounts less than 0.1 cc. In 100 samples of bottled raw milk they found no colon organisms in 30 per cent of the 10 cc. samples.

Moore and Fuller (17), in comparing the total and the colon count of milk found that the majority of farms producing milk free of colon organisms likewise had lower counts than the farms from whose milk colon organisms were isolated, yet there was no direct relationship between the score placed on the farm and the presence of colon organisms in the milk or between the score and average bacterial count. Davis (18) concludes that the *B. coli* test is of no value as an indicator of gross contamination, but does serve to indicate faulty production and unsterilized utensils. He found that no growth of these organisms occurred in milk held below 10° C.

Chilson, Yale and Eglinton (19) observed that the test for the colon group "should supplement and not supplant the standard plate count," in detecting contamination of pasteurized milk, since "there were instances in which one or the other of the methods indicated recontamination when the other did not."

EXPERIMENTAL

Three hundred thirty-one samples of raw milk were examined for total bacterial count using Standard Methods of Milk Analysis procedures (1934), the coli-aerogenes being isolated on five liquid and nine solid media in groups of two liquid and two solid media with 50 samples in each group, as reported in a previous paper. In addition 50 samples were plated in neutral red bile agar in 1 cc. amounts and in dehydrated tryptone glucose milk medium of Bower and Hucker (20), duplicate platings being made in each case. The latter medium was incubated for 48 hours at 32° C. It has been shown that higher counts may be obtained on a modified medium at lower temperatures, than can be obtained on standard agar at 37° C. These results have been confirmed in this laboratory.

TABLE 1

Prevalence of coli-aerogenes in 331 samples of raw milk grouped on basis of total count on standard agar

TOTAL COUNT	NUMBER OF COLI-AEROGENES PER CC.			
	0	1–9	10–99	100
	per cent	per cent	per cent	per cent
Under 10,000	36.4	32.8	23.8	7.0
Over 10,000	21.1	8.0	28.6	42.3
Under 50,000	31.8	22.6	27.9	17.7
Over 50,000	16.2	8.1	20.3	55.4
	Number of Escherichia per cc.			
Under 10,000	53	25	18	4
Over 10,000	47	10	17	26
Under 50,000	53	18	19	10
Over 50,000	37	10	16	37
	Number of Aerobacter per cc.			
Under 10,000	80	12	5	3
Over 10,000	70	4	11	15
Under 50,000	74	10	10	6
Over 50,000	75	3	4	18
	Number of Intermediates per cc.			
Under 10,000	82	12	4	2
Over 10,000	71	3	11	15
Under 50,000	77	10	9	4
Over 50,000	70	2	6	22

Thirty-one samples of pasteurized milk and 25 samples of milk meeting the requirements of certified milk were examined, the coli-aerogenes group being determined on two liquid and three solid media. The generic separation was made in accordance with the methods suggested in the 7th edition of Standard Methods of Water Analysis.

RESULTS

Table 1 shows the results obtained with 331 samples of raw milk, which were separated into different grades based on total count and the number of all colon organisms and of the different members of this group. The percentage of samples negative for colon organisms does not vary significantly in samples under or above 10,000 total count; however, 69.2 per cent of the samples with counts under 10,000 showed less than 10 colon organisms per cc., while 29.1 per cent of the samples having a total above 10,000 contained this number of colon organisms. It must be remembered that 9.4 to 22 cc. of milk were used for inoculation, so that the actual number of coli-aerogenes per cc. might be lower than is indicated.

On comparing the various members of the coli-aerogenes group with the total counts, it appears that the group as a whole gives a better correlation with total count than any of the individual members.

Of these samples 27.9 per cent were negative for colon in 0.01 cc., 19.1 per cent were positive in 1 cc., 26 per cent were positive in 0.1 cc. and 27 per cent were positive in 0.01 cc. If the 1 cc. positives are eliminated, 47.0 per cent of the samples would be negative in all amounts, thus corresponding closely with the results of Malcolm and of Klimmer, Haupt and Borches, as shown in Table 2.

TABLE 2

Percentage of coli-aerogenes found in various amounts of raw milk by different investigators

	MALCOLM (9)	KLIMMER, HAUPT & BORCHES (15)	PRESENT DATA
Negative in 0.1 cc.	48.3	57.0	47.0
Positive in 0.1 cc.	21.4	23.5	26.0
Positive in 0.01 cc.	14.0	10.0	27.0
Positive in 0.001 cc.	16.3	23.5	.....

The average total count of the colon-positive samples was 140,500 per cc. or 2.9 times that of the colon-negative samples, which was 48,270. Malcolm, in the investigation cited, found an average count of 160,577 in coliform-positive and 25,295 per cc. in the coliform-negative samples, the positive samples having 6.3 times as many bacteria per cc. as the negative

The 50 samples of raw milk, plated on neutral red bile and tryptone glucose milk medium, showed a pronounced relationship between colon titre

and total count, however, the number of samples is not sufficient for the drawing of definite conclusions. The results given in Table 3 show 93.5 per

TABLE 3

Prevalence of coli-aerogenes in fifty samples of raw milk of different total counts on glucose tryptone agar at 32 C.

TOTAL COUNT	NUMBER OF COLI-AEROGENES PER CC.			
	0	1–9	10–99	100
	per cent	per cent	per cent	per cent
Under 10,000	93.5	6.5	0	0
Over 10,000	22.2	77.8	0	0
Under 50,000	85	15		
Over 50,000	0	100		

cent of the samples, with a total count of under 10,000, to be negative in all amounts while 6.5 per cent were positive in 1 cc. amounts. The samples with a total count of over 10,000 showed 22.2 per cent to be negative in all amounts and 77.8 per cent positive in 1 cc. amounts. The average count of the colon-negative samples was 6,650; that of the colon-positive 80,000 or 12 times as many. The logarithmic average of the colon-negative samples, computed by the method of the U. S. Public Health Service Milk Ordinance and Code (21), was 5,000; of the positive 45,000 or 9 times as many.

Thirty-eight per cent of the 31 samples of pasteurized milk contained colon organisms with an average total count of 17,900 while 62 per cent were negative and had an average count of 4,171, the positive samples having 4.3 times as many bacteria per cc. All of the positive samples were obtained from one plant where coli-aerogenes organisms were found from the cooler, bottler, and from the bottled milk, this plant had an exceptionally high bacterial count even in the pasteurizing vat. The two other plants studied showed no colon organisms in the bottled milk and the count never exceeded 700 per cc. The frequency of the colon organism in relation to total count is shown in Table 4.

TABLE 4

Prevalence of coli-aerogenes in 31 samples of pasteurized milk of different total counts on standard agar

TOTAL COUNT	NUMBER OF COLI-AEROGENES PER CC.			
	0	1–9	10–99	100
	per cent	per cent	per cent	per cent
Under 1,000	100			
1–10,000	40	40	10	10
Over 10,000	30	40	30	

The 25 samples of "certified" milk gave an average total count of 1,686 for the colon-positive samples which was 1.6 times as great as 1,017, the

average count of the colon-negative samples. No colon organisms were found in 0.1 cc. of these samples. Since no samples were examined in which the total count exceeded 10,000, comparison of Kline's (8) results could not be made.

DISCUSSION

It is felt from the results obtained with pasteurized milk that the standard proposed by Swenarton, "that not more than 20 per cent of 0.1 cc. portion shall show *E. coli*" or that "positive results shall not occur in more than 10 per cent of 0.1 cc. portions when 10 or more samples are examined," is not unduly stringent. It would seem entirely possible for this standard to be met if based on the results of 1 cc. portions, which is the suggestion of McCrady and Langevin (14), who concluded that "properly pasteurized milk should not contain coli-aerogenes bacteria in 1 cc. portions, in more than 10 to 20 per cent of tests." Slack and Maddeford (16) found that colon bacilli in 0.1 cc. indicates recontamination and that absence in 50 cc. may "reasonably be expected." A correlation of 38 per cent between low count and absence of colon bacilli was found by these investigators. It is believed that from these results and from those of other investigators, an accurate test for coli-aerogenes organisms in pasteurized and in certified milk would be a valuable aid in determining the sanitary quality of the milk.

This is well emphasized in the present investigation of the one pasteurizing plant from which samples positive for colon were obtained. As indicated, they were found present in samples taken from the milk at every point after it had passed over the cooler, at the same time no increase in total bacterial count was observed. Thus the colon examination indicated faulty methods or equipment while the total count failed to show any contamination occurring.

From the work with raw milk it is believed that no standard for this grade can at the present time be set, however, it would seem possible that reasonably careful production should yield a product having fewer than 10 colon organisms per cc. in 70 to 80 per cent of examined samples and that in no instance should 100 per cc. be exceeded. Additional work using tryptone glucose agar or other modified agar at 32° C. for the comparison of total count and presence of the coli-aerogenes group is desirable.

SUMMARY

A comparison of the total count on standard agar and presence of colon organisms showed the colon-positive samples of raw milk to have a total count of 2.9 times that of the colon-negative. Less than 10 colon organisms per cc. were found in 69.2 per cent of samples with a total count of less than 10,000 bacteria per cc. No pasteurized samples with counts under 1,000 contained colon organisms, the count of colon-positive samples being 4.3

times higher than colon-negative samples. No colon organisms were found in 0.1 cc. of "certified" milk.

Total bacterial counts made on tryptone glucose milk medium at 32° C. gave excellent correlation with colon titre, since 93.5 per cent of samples with a total count under 10,000 were negative for colon organisms. The average count of colon-positive samples was 12 times higher than that of colon-negative samples, being 6,650 and 80,000 per cc. respectively.

The presence of the coli-aerogenes group in raw milk of high bacterial count, would seem to be of little significance, in milk of low count the number of colon bacteria might be limited to the presence in 1 cc. amounts in 70–80 per cent of samples examined. In order to determine the sanitary condition under which milk is produced, further work to determine a standard for these organisms is desirable.

The presence of coli-aerogenes organisms in 1 cc. of pasteurized milk in 10 to 20 per cent of samples would seem sufficient to indicate contamination, which, as is shown in this investigation, might not be indicated by total bacterial counts. This test in pasteurized milk is particularly desirable.

The present standards for certified milk in respect to colon content are quite lenient.

Lactose taurocholate agar was found unsatisfactory in previous experiments in this series so that the adoption of a more satisfactory method for the detection of these organisms would be desirable and if this is realized a more stringent standard would seem possible. The value of a test to indicate contamination in a milk to be consumed raw can not be too highly stressed.

REFERENCES

(1) Beavens, E. A. The Escherichia-Aerobacter Group as an Index to Proper Pasteurization. Journal of Dairy Science, 13: 91, 94. 1930.

(2) Minkin, J. L., and Burgwald, L. H. A Study of Escherichia-Aerobacter Organisms in Pasteurized Milk. Journal of Dairy Science, 18: 474. 1935.

(3) Stark, C. N., and Patterson, Mary Caroline. The Heat Resistance of Colon Organisms in Milk. Paper presented at the Annual Meeting of the Am. Dairy Sci. Assoc., 1936.

(4) Ayres, S. H., Cook, L. B., and Clemmer, P. W. The Significance of the Colon Bacilli in Milk. Abst. of Bact., 1: 52. 1917.

(5) Hunter, O. W. The Colon-Aerogenes Group of Milk. Journal of Dairy Science, 2: 108. 1919.

(6) Finkelstein, R. Occurrence of the Colon-Aerogenes Group of Organisms in Raw and in Pasteurized Milk, and its Significance. Journal of Dairy Science, 2: 460. 1919.

(7) Swenarton, J. C. Can B. coli be Used as an Index of the Proper Pasteurization of Milk. J. Bact., 13: 419. 1927.

(8) Kline, E. K. The Colon Group of Bacteria in Milk. 19th. Ann. Report Internat. Assoc. Dairy and Milk Inspectors, 68. 1930.

(9) Malcolm, J. F. The Occurrence of Coliform Bacteria in Milk. J. Dairy Res., 5: 15. 1933.

(10) MUNCHBERG, F. The Hygienic Estimation of Pasteurized Milk. Wien. tierarztl Mschr. 20: 278. 1933.

(11) SHERMAN, J. M., AND WING, HELEN U. The Significance of Colon Bacteria in Milk, with Special Reference of Standards. JOURNAL OF DAIRY SCIENCE, 16: 165. 1933.

(12) BARKWORTH, H. A Statistical Examination of the Interrelationship and Variability of Plate Count, Presumptive Coliform Content and Keeping Quality of Raw Milk. J. Dairy Res., 6: 26. 1935.

(13) MOLDAVAN, A. A Modified Technic for the Detection of the Escherichia-Aerobacter Group in Milk. Am. J. Pub. Health, 25: 1032. 1935.

(14) MCCRADY, M. E., AND LANGEVIN, E. M. The Coli-Aerogenes Determination in Pasteurization Control. JOURNAL OF DAIRY SCIENCE, 15: 321. 1932.

(15) KLIMMER, M., HAUPT, H. U BORCHES, F. Uber der Vorkommen und die Bestimmung der Coli und Aerogenes Bakt. der Milch. Milch Forsch., 9: 236. 1930.

(16) SLACK, A. J., AND MADDEFORD, C. W. The B. coli Content of Raw and Pasteurized Milk. Canadian Pub. Health J., 23: 574. 1932.

(17) MOORE, H. C., AND FULLER, J. M. Significance of Colon Organisms in Raw Milk. Tech. Bul. 57, Univ. of New Hampshire Agri. Exp. Sta. 1934.

(18) DAVIS, G. O. The Significance of (a) Acid Fast Bacteria; (b) Bacillus coli in Milk. Vet. Record. 13: 1046. 1933.

(19) CHILSON, W. H., YALE, M. W., AND EGLINTON, R. Detecting Recontamination of Pasteurized Milk by Bacteriological Methods. JOURNAL OF DAIRY SCIENCE, 19: 337. 1936.

(20) BOWER, C. S., AND HUCKER, G. J. The Composition of Media for the Bacteriological Analysis of Milk. Tech. Bul. 228. New York Agri. Exp. Sta., Geneva. 1935.

(21) United States Public Health Service Milk Ordinance and Code. Treasury Department, U. S. Public Health Service, August, 1934.

CHANGES IN WEIGHT OF NEW BORN DAIRY CALVES AS RELATED TO THE METHOD OF FEEDING*

C. L. COLE
North Central Experiment Station, University of Minnesota, Grand Rapids, Minnesota

INTRODUCTION

The fact that new born human babies lose part of their birth weight the first few days following birth has been established for some time. Members of the medical profession have expressed an interest in the behavior of dairy calves for the corresponding period of time. The question as to whether or not calves lose weight and whether or not the dairyman does anything about it has been asked. With this question before us a large number of calves have been weighed in an effort to furnish a satisfactory answer and to make recommendations as to the best method of starting new born dairy calves.

EXPERIMENTAL PROCEDURE

The calves used in this experiment were both purebred Guernseys and grades with at least seven crosses of purebred Guernseys. Both males and females were used.

Each calf was weighed immediately following birth and at eight o'clock each morning thereafter until the age of fourteen days. The calves were divided into three separate groups. The thirteen calves of the first group were taken from their dams immediately after birth without nursing. A small amount of colostrum milk was given at once and thereafter they were fed each morning and evening from their dam's milk. The amount fed each calf ranged from three to six pounds per day depending on the weight of the calf. The amount of milk fed per day was increased as rapidly as the calf would take it until the amount was equal to ten per cent of the body weight.

The twenty-six calves in the second group were left with their dams for a period of forty-eight hours. They were then separated from their dams and food was withheld for a period of twenty-four hours at the end of which time they were started on hand feeding of whole milk at the same rate as group one. The third group contained fourteen calves which were left with their dams for a period of ninety-six hours and then cared for in exactly the same manner as group two.

A summary of the results obtained from the three groups is given in Table 1. The data for the human babies was obtained through the courtesy

Received for publication September 17, 1936.
Paper No. 1435, Scientific Journal Series, Minnesota Agricultural Experiment Station.
* The author wishes to acknowledge the assistance of the late Dr. Clarence H. Eckles, who conceived and helped plan this investigation.

TABLE 1
Changes in weight

TIME AFTER BIRTH-DAYS	BIRTH	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Weight Pounds	Group I Calves Removed from Dams at Birth (Average for 13 calves).														
	65.653	64.73	64.692	65.73	66.846	68.23	69.538	70.73	71.807	73.23	74.50	75.884	77.384	78.961	80.384
Weight Pounds	Group II Calves Left with Dams 48 hours (Average for 26 calves).														
	64.98	67.269	67.634	65.153	65.173	65.615	66.326	67.538	68.557	69.307	69.557	70.346	70.73	71.307	71.807
Weight Pounds	Group III Calves Left with Dams 96 hours (Average for 14 calves).														
	65.607	67.50	67.785	68.107	69.178	68.25	66.535	66.428	66.785	67.071	67.50	67.928	68.678	69.214	69.821
Weight Pounds	Human Babies (Average for 21 Babies).														
	7.813	7.711	7.401	7.22	7.15	7.178	7.231	7.291	7.533						

of the management of the Itasca County Hospital, Grand Rapids, Minnesota, from the records of twenty-one babies born at the hospital. They are shown for only eight days because nearly all the babies were removed from the hospital at the end of that period. The figures are given for the sole purpose of comparison as they probably represent fairly well the typical changes of weight in the human species following birth.

DISCUSSION OF RESULTS

A study of Table 1 shows that the calves of Group I suffered a daily loss for the first two days, probably due to the small amount of milk they would take. There was a continuous daily gain for the remaining fourteen days. The loss was so slight for the first two days that one day of gain compensated for the entire loss and could probably be accounted for largely by the difference in fill.

The table for Group II shows that the calves gained daily for the first two days and then lost on the third day which represents the time food was withheld after the calves were separated from their dams. On the fourth day, after they had received milk by hand feeding the preceding twenty-four hours, the calves started to gain weight and there was a steady daily gain for the balance of the fourteen day period. In order to check this loss on the third day and make certain that it was induced by the period of fasting preceding hand feeding, the calves of Group III were left with their dams for the ninety-six hour period.

The table for Group III shows about the same average gain during the first two days as for Group II and a continued gain for the next two days, while the calves were nursing their dams. On the fifth day, which was immediately after the twenty-four hour period of fasting, a decline occurred. The calves also lost weight on the sixth and seventh day and then made a steady gain each day during the balance of the experiment.

In both Groups II and III there was a steady gain immediately following birth until the calves were removed from their dams and subjected to a twenty-four hour fasting period.

The fact that the calves failed to take enough milk during the period of learning to drink probably accounts for the loss of weight in Group I during the first days. That the calves in Group III continued to lose weight for a period of three days after taking them from their dams can only be accounted for by the fact that they would not drink as much milk at the start as the calves in Group II. Their average loss exceeded Group II by only .269 lbs. even though it occurred over a longer period.

The important fact brought out in this study is the decided advantage in gain shown at the end of the fourteen day period by the calves not allowed to nurse, over those that nursed, also the advantage of those left with their dams forty-eight hours over those left with their dams ninety-six

hours. At birth Group I averaged .673 lbs. more than Group II and .046 lbs. more than Group III but at the end of the fourteen day period they averaged 8.577 pounds more than Group II and 10.563 pounds more than Group III giving them an advantage in gain of 7.904 pounds and 10.517 pounds over Groups II and III respectively.

CONCLUSIONS

1. Dairy calves do not suffer a natural loss in weight immediately following birth as indicated by the immediate gain made by all calves when allowed to nurse their dams.

2. Dairy calves seem to get a better start during the first fourteen days when removed from their dams immediately after birth and started on hand feeding.

3. The calves in this experiment that were not allowed to nurse were much easier to teach to drink than those that nursed their dams. The rapidity and ease with which the calves in this experiment learned to drink was in direct proportion to the length of time they were allowed to nurse.

4. The average birth weight of the fifty-three Guernsey calves used in this experiment was 65.3 pounds.

American Dairy Science Association Announcements

ANNUAL MEETING

MONDAY, JUNE 21, 1937

1 P. M.–9 P. M.—Registration, Dairy Industry Building, Agricultural College Campus.

TUESDAY, JUNE 22, 1937

8 A. M.–9 P. M.—Registration, Dairy Industry Building, Agricultural College Campus.
9–10:30 A. M.—Extension Section, Room 303, Dairy Industry Building.
10:30–10:40—Rest period.
10:40–12:00—Extension Section, Room 204, Dairy Industry Building.
12:00– 1:00—Lunch, Agricultural College Cafeteria.
1:00– 4:00—Demonstration—Reproduction, Dairy Barn.
1:00– 4:00—Ice Cream Scoring, Dairy Industry Building.

7:00– 8:00—General Meeting, Temple Theater.
8:15—General Get-together, Lawn, Carrie Belle Raymond Hall.

WEDNESDAY, JUNE 23, 1937

8:00–12:00—Registration, Dairy Industry Building, Agricultural College Campus.
8:00– 9:00—Sectional Committee Meetings:
Production, Room 206, Dairy Industry Building.
Manufacturing, Animal Husbandry Hall.
Chemical Methods of Analysis, Animal Husbandry Hall.
Extension, Animal Husbandry Hall.
9:00–12:00—General Session, College Activities Building, Agricultural College Campus.
12:00– 1:00—Lunch, Agricultural College Cafeteria.
1:00– 2:10—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing, Room 303, Dairy Industry Building.
2:10– 2:20—Rest period.
2:30– 4:00—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing, Room 303, Dairy Industry Building.
4:00– 5:00—Section Committee Meetings:
Production, Room 206, Dairy Industry Building.

Manufacturing, Animal Husbandry Hall.
Extension, Animal Husbandry Hall.
Visit Morrill Hall and Capitol.

8:00—Get-together, social time, Carrie Belle Raymond Hall, or Shrine Country Club.
Entertainment features.

THURSDAY, JUNE 24, 1937

8:00—Extension Exhibits open.
8:00– 9:50—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing Section, Room 303, Dairy Industry Building.

9:50–10:00—Rest period.
10:00–12:00—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing Section, Room 303, Dairy Industry Building.
12:00– 1:00—Complimentary Lunch, Dairy Husbandry Department.
1:00– 2:00—Sectional Business Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing, Room 303, Dairy Industry Building.
Extension, Room 204, Dairy Industry Building.
2:00– 3:00—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing, Room 303, Dairy Industry Building.
Extension, Room 204, Dairy Industry Building.
3:00– 4:00—Dairy Education and Teaching Problems, Room 301, Dairy Industry Buildings.

6:30—Subscription Banquet, Shrine Country Club.
Followed by entertainment.

FRIDAY, JUNE 25, 1937

8:00– 9:00—General Business Session, Room 301, Dairy Industry Building.
9:00–12:00—Sectional Meetings:
Production, Room 301, Dairy Industry Building.
Manufacturing Section, Room 303, Dairy Industry Building.

NOMINATIONS FOR THE BORDEN AWARDS FOR WORK IN THE PRODUCTION AND PROCESSING FIELDS FOR 1937

The recipients of the two annual Borden awards, each consisting of $1000 and a gold medal, for the most meritorious research in the field of dairy cattle production and in the processing field, will be selected by the awards committee of the American Dairy Science Association. Send your nomination for these honors to the Secretary of the American Dairy Science Association, Department of Dairy Technology, University of Ohio, Columbus, Ohio, so that it will reach him not later than March 1, 1937. The awards will be made for (1) the most outstanding research work in the production field, breeding, feeding, farm sanitation or quality production, and other phases of production work, and (2) for outstanding research in the processing field such as improvement in equipment or methods in the handling of milk or cream and the production of milk products. Nominations should be made for those whose work has been completed and published during the five-year period ending December 31, 1936.

Those who are eligible for consideration for the awards: Any living citizen of the United States or Canada. There are no limitations as to age or sex.

Since Borden awards are also being made for meritorious work in basic science as related to, or as it affects the Dairy Industry; for work in basic research on vitamins or other nutritional aspects of milk products; for work in the practical application of the findings in nutritional research; and for work in public health as related to the Milk Industry, these awards to be administered by other scientific societies, these phases of research work as applied to the dairy industry should not be considered in making your nominations for research in the production and the processing fields.

Each member of the American Dairy Science Association is entitled to make nominations for these awards. The form for making nominations is as follows:

TO THE SECRETARY, AMERICAN DAIRY SCIENCE ASSOCIATION

I, the undersigned member of the American Dairy Science Association nominate .....
for the Borden award for 1937 in the field of production or processing for his contribution entitled .....
.....
and delivered before .....
or published in .....
(Give details of publication references and if possible enclose copies.)
Signed: .....

THE 1936 COLLEGIATE STUDENTS' NATIONAL DAIRY CATTLE JUDGING CONTEST

The 1936 students' national contest in judging dairy cattle was held at the National Dairy Show, Dallas, Texas, October 12, 1936. The cattle in each ring were placed by a special committee consisting of coaches with teams in the contest. The placing of the different animals depended upon the average placing of each animal by all of the coaches who served as judges.

Twenty teams competed in the contest, judging rings of the five dairy breeds.

Five high individuals on each breed

Place	*Contestant's Name*	*College Represented*
	Ayrshire	
1	H. R. Hofstrand	Iowa
2	Martin Baugher	Missouri
3	David Carder	Nebraska
4	Chas. Beer	Kansas
5	H. N. Haferbecker	Wisconsin
	Brown Swiss	
1	E. J. Preslik	Wisconsin
2	Lawrence Johnson	Michigan
3	H. N. Haferbecker	Wisconsin
4	Elmer Dawdy	Kansas
5	David Carder	Nebraska
	Guernsey	
1	R. O. Bergstrom	Wisconsin
2	H. N. Haferbecker	Wisconsin
3	E. J. Preslik	Wisconsin
4	Ivan Bowman	Nebraska
5	S. J. Taylor	Texas A. & M.
	Holstein	
1	Brooks Naylor	Minnesota
2	E. J. Preslik	Wisconsin
3	Charles Beer	Kansas
4	J. L. Leslie	Ontario
5	J. S. Taylor	Texas A. & M.
	Jersey	
1	Lawrence Johnson	Michigan
2	Clayton Chappell	Tennessee
3	Goodwin Soustegaard	Minnesota
4	Charles Beer	Kansas
5	Noel Ralston	Missouri

Five high individuals—all breeds

Place	Contestant's Name	College Represented
1	Preslik, E. J.	Wisconsin
2	Soustegaard, Goodwin	Minnesota
3	Naylor, Brooks	Minnesota
4	Haferbecker, H. N.	Wisconsin
5	Teague, F. M.	Iowa

Five high teams—by breeds

Place	Team	Place	Team
	Ayrshire		*Brown Swiss*
1	Iowa	1	Wisconsin
2	Kansas	2	Nebraska
3	Nebraska	3	Kansas
4	South Dakota	4	Cornell
5	Minnesota	5	Texas Tech.
	Guernsey		*Holstein*
1	Wisconsin	1	Minnesota
2	Minnesota	2	Ontario
3	Michigan	3	Nebraska
4	Nebraska	4	Texas A. & M.
5	Kansas	5	Wisconsin

Place	Team
	Jerseys
1	Texas A. & M.
2	Kansas
3	Minnesota
4	Missouri
5	Michigan

Team standing—all breeds

Place	Team	Points Scored	Place	Team	Scored Points
1	Minnesota	4347.3	11	Tennessee	4119.3
2	Kansas	4313.9	12	Ontario	4047.0
3	Wisconsin	4312.6	13	Oregon	3962.3
4	Nebraska	4311.9	14	Ohio	3937.9
5	Texas A. & M.	4235.2	15	South Dakota	3902.0
6	Texas Tech.	4181.3	16	New Mexico	3840.9
7	Iowa	4181.1	17	Purdue	3719.9
8	Cornell	4159.1	18	Virginia	3689.7
9	Michigan	4156.4	19	Arkansas	3667.2
10	Missouri	4129.8	20	Mississippi	3419.1

VOLUME XX FEBRUARY, 1937 NUMBER 2

JOURNAL OF DAIRY SCIENCE

Published by the

AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Ohio State University, Columbus, Ohio, Sec-Treas.

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

Published in cooperation with

INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

Editorial Committee

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
J. C. HENING, Geneva, New York, A. D. S. A.
L. W. BASS, New York, New York, I. A. I. C. M.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
R. C. FISHER, Waterbury, Connecticut, I. A. M. D.
W. H. MARCUSSEN, New York, New York, I. A. M. D.

A. C. DAHLBERG, Editor, Geneva, New York

CONTENTS

Bacteriology
Butter
Cheese
Chemistry
Concentrated and dry milk
Food value
Ice cream
Milk
Miscellaneous

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Fay, A. C., Holm, G. E., Marquardt, J. C., Phillips, C. A., and Tracy, P. H.

ABSTRACTORS

Babcock, C. J.
Barkman, J. O.
Bartlett, J. W.
Becker, R. B.
Bendixen, H. A.
Bennett, F. W.
Bird, E. W.
Burgwald, L. J.
Burri, R.
Brueckner, H. J.
Burke, A. D.
Bushnell, L. D.

Carpenter, D. C.
Clevenger, W. L.
Coie, W. C.
Coulter, S. T.
Cunningham, O. C.
Cunningham, W. S.

Dahlberg, A. C.
Darnell, A. L.
Demeter, K. J.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.
England, C. W.
Erb, J. H.

Fabian, F. W.
Frayer, J. M.
Frazier, W. C.
Fuller, J. M.

Geipi, A. J.
Golding, N. S.
Goss, E. F.
Greenbank, G. R.
Guthrie, E. S.

Hansen, Arne
Hening, J. C.
Herrington, B. L.
Herzer, F. H.
Holdaway, C. W.
Horrall, B. E.
Hucker, G. J.

Jacobson, C. O.
Jensen, Chris
Johnson, A. H.

Keith, J. I.
Knight, D.
Krauss, W. E.

LaMaster, J. P.
Leighton, A.
Lindquist, H. G.
Locke, R. B.
Lucas, P. S.

Mack, M. J.
Macy, H.
Marquardt, J. C.
Martin, W. H.
Maynard, L. A.
Morris, A. J.
Mudge, C. S.
Mueller, W. S.

Nair, J. H.
Nelson, D. H.
Nelson, J. A.

Overman, O. R.

Palmer, C. C.
Parfitt, E. H.
Price, W. V.

Reid, W. H. E.
Richardson, G. A.
Riddell, W. H.

Sommer, H. H.
Stark, C. N.
Swope, W. D.

Theophilus, D. R.
Thomsen, L. C.
Thurston, L. M.
Tobey, J. A.
Totman, C. C.
Trout, G. M.
Tuckey, S. L.

Webb, B.
Weckel, K. G.
Wilster, G.
Wylie, C. E.

Yale, M. W.

JOURNALS

American Creamery and Poultry Produce Review
American Journal of Diseases of Children
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Certified Milk

Dairy Produce Review
Dairy World
Deutsche Molkerei Zeitung

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Industry
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of American Chemical Society
Journal of American Medical Association
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Experimental Medicine
Journal of General Physiology
Journal of Infectious Diseases
Journal of London Chemical Society
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry

Kaseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry

Milk Plant Monthly
Molkerei Zeitung

National Butter and Cheese Journal

Pacific Dairy Review

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zentralblatt für Bacteriologie

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers
International Association of Milk Inspectors

National Dairy Experiment Station, Hillerod, Denmark
National Institute for Research in Dairying, Reading, England

New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BACTERIOLOGY

39. Infectious Bovine Mastitis. Report on a Control Program Based on Segregation of Infected Animals. W. N. Plastridge, E. O. Anderson, F. J. Weirether and R. E. Johnson, Storrs Agr. Exp. Sta., Storrs, Conn. Journal of Dairy Science *19*, 10, p. 641, Oct., 1936.

The incidence of streptococci mastitis was greatly reduced in herds of dairy cows segregated and handled on the basis of the extent of the infection.

A.C.D.

40. Observations Relative to the Staining of Micro-organisms Previously Heated in Milk. G. Guittonneau and Jeanne Brigando, National Lab. of the Dairy Industry, Paris, France. Le Lait *16*, 156, p. 577, June, 1936.

In studying the staining properties of certain lactic organisms previously submitted to a vigorous heat treatment in milk, it was found that the organisms acquired an abnormal resistance to staining by most of the usual techniques such as the gram stain, the classic treatment with aqueous methylene blue, gentian violet, the blue of China and various coloring acids. Some staining can be obtained by the usual techniques by prolonging considerably the period of contact with the dye (10 to 12 hours instead of a few minutes). The staining properties of the organisms in the heated milk become normal again on treating with dilute acid. With such vigorous dyes as the phenic fuchsine of Ziehl, there is scarcely any change in staining properties as a result of heating the milk which contains the lactic organisms. On substituting *B. subtilis* or *B. megatherium* for the lactic organisms in the milk, there is no difference in staining properties as a result of heating the milk. In suspension in milk without subsequent heating, all the organisms mentioned take the dyes easily by the classic procedure, especially by simple treatment with aqueous methylene blue. On treating with methylene blue the lactic organisms appear to be surrounded by a corona. An analogous corona cannot be discerned under the same conditions with *B. subtilis* nor with *B. megatherium*. After staining with the phenic fuchsine of Ziehl and with the acetic acid differentiation, clear coronas are on the contrary visible in all cases.

The particularities of staining that have just been described were found again to a more or less attenuated degree in a series of tests where milk was replaced by pseudo-solution of a complex calcium caseinophosphate. The

resistance to staining with methylene blue did not manifest itself, however, in the case of the lactic organisms heated in lacto-sera where the caseino-phosphate complex was eliminated with rennin. The facts which have been given suggest that a chemical or physical reaction intervenes between the surface layer of the membrane of the organism or the capsule and the other part, a colloidal material from the medium in the nature of a phospho-caseinate. This reaction already beginning at ordinary temperature increases under the influence of heat and terminates or not depending on the case, with the formation of a protective envelope resistant to certain dyes. The nature of the capsulary surfaces in contact with the phospho-caseinates, and the type and cultural state of the bacterial strain submitted to the heat test in the milk will no doubt condition the appearance of the forms of microbiological protection against the action of methylene blue. However, the staining phenomena that have been described may be attribued to other hypotheses—notably to selective adsorption of the dye as modified by the action of heat. A.H.J.

CHEESE

41. A Study of the Egyptian Cheese Called "Mich." G. El-Geriany Mostafa. Le Lait *16*, 155, p. 485, May, 1936.

To curdled skimmilk which has been stirred are added cylinders of curd (from cow or buffalo milk) 6 cm. long by 4 cm. in diameter. The mixture of curd and curdled milk is contained in a clay jar which is sealed with straw and mud. An anaerobic fermentation sets in and at the end of a month the cheese is ready for consumption but the fermentation may proceed for as long as a year. The finished cheese is yellowish white in color, has a piquant taste and the odor is strong due to the high volatile acidity. Bacteriological examination of cold cheese indicates that it is very low in micro-organisms. On progressive aging of the cheese for 1 week, 1 month, 5 months, and 8 months, the pH values were 4.0, 4.5, 4.96, and 5.0 and the volatile acidities were 20°, 22°, 46°, and 60° Soxhlet-Henkel, respectively. A.H.J.

42. Studies on the Emulsifying Salts Used in Processed Cheese. Hugh L. Templeton and H. H. Sommer, College of Agr., Madison, Wisconsin. Journal of Dairy Science *19*, 8, p. 561, Aug., 1936.

The authors concluded that sodium citrate was probably superior to other salts commonly used to emulsify cheddar cheese. A.C.D.

43. The Bacteriology of Swiss Cheese. W. C. Frazier, H. F. Long, and Wm. T. Johnson, Jr., Res. Lab., Bureau of Dairy Industry, Washington, D. C. Journal of Dairy Science *19*, 8, p. 535, Aug., 1936.

Streptococcus thermophilus cultures improved the quality of Swiss cheese when added to milk with a methylene blue reduction period of more than 5 hours but did not improve the quality when the reduction time was less. A.C.D.

Other abstracts of interest are numbers 45, 48.

CHEMISTRY

44. The Phospholipids in Milk. IV. Their Chemical Nature and Their Distribution Among Some Milk Products. GEO. E. HOLM, P. A. WRIGHT, AND E. F. DEYSHER, Divis. of Dairy Res. Lab., Bureau of Dairy Industry, Washington, D. C. JOURNAL OF DAIRY SCIENCE *19*, 10, p. 631, Oct., 1936.

The phospholipids of milk were found to be lecithin, cephalin, and sphingomyelin in the ratio 8.4, 4.5, and 1. The phospholipids contain 4 per cent of phosphorus. Their distribution in dairy products tends to follow the milk fat. A.C.D.

45. Effect of Salts on the Solubility of Casein and Paracasein. PAUL F. SHARP AND T. J. MCINERNEY, Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y. JOURNAL OF DAIRY SCIENCE *19*, 8, p. 573, Aug., 1936.

Sodium chloride and sodium iodide shifted the solubility of paracasein to a more alkaline reaction and make pH 5.5 to 6.0 the region for maximum peptizing action of salt to obtain greatest smoothness in texture. A.C.D.

46. Accumulation of Protein in the Foam of Skimmilk. PAUL F. SHARP, ROBERT P. MYERS, AND E. S. GUTHRIE, Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y. JOURNAL OF DAIRY SCIENCE *19*, 10, p. 655, Oct., 1936.

The proteins of skimmilk accumulated in the foam in the same proportion in which they occurred in skimmilk. A.C.D.

CONCENTRATED AND DRY MILK

47. The Determination of Moisture in Powdered Milk by the Toluol Distillation Method. E. C. THOMPSON AND R. S. FLEMING, The Borden Co., New York and Syracuse, N. Y. JOURNAL OF DAIRY SCIENCE *19*, 8, p. 553, Aug., 1936.

The Toluol Distillation Method of determining the moisture content of milk powder permitted the use of a large sample, the results were secured within an hour, gave accurate results, and was well adapted to plant control work. A.C.D.

48. The Examination by English Specialists of the Synthetic Wool Prepared in Part from Casein. G. GENIN, Paris, France. Le Lait *16*, 155, p. 510, May, 1936.

A comparison is made of some of the properties of genuine wool and the Italian wool said to be made of casein and formaldehyde. On soaking in water, the diameter of the fibres increases to about the same extent in both cases, viz., about 7 per cent. Longitudinally, however, genuine wool increased 1 to 2 per cent while the synthetic wool made from casein increased 7 to 15 per cent. If the synthetic fibre is submitted to a strain after it has been in the water, it elongates considerably, often to twice its original length and the fibre breaks when a force is applied that is only one fourth to one eighth that which is required to break a similarly treated genuine wool fibre of the same diameter. The synthetic fibres were much less resistant to trypsin than were the genuine wool fibres. The synthetic fibres had much the same outward appearance as the genuine wool fibres but had a slightly more rough feeling. Under the microscope, the synthetic fibres were readily distinguishable from the genuine wool fibres. On suspending the fibres in an alkaline solution, about 10 times as much material dissolved from the synthetic fibres as from the genuine wool fibres. The synthetic fibre appeared only to serve satisfactorily in a mixture with more stable fibres of cotton or of viscose for the fabrication of special tissues destined to fill particular needs in the Italian economic situation. It does not appear that the synthetic wool fibres will replace genuine wool fibres except in special cases. A.H.J.

Other abstracts of interest are numbers, 44, 45, 46, and 61.

FOOD VALUE

49. Vitamin A Activity of Third Cutting Alfalfa Hay as Affected by Methods of Curing. ELLA WOODS, F. W. ATKESON, HARRY WELLHOUSEN, AND R. F. JOHNSON, Idaho Agr. Exp. Sta., Moscow, Idaho. JOURNAL OF DAIRY SCIENCE *19*, 9, p. 581, Sept., 1936.

The vitamin A content of alfalfa hay varied with the method of curing and decrease during storage. A.C.D.

ICE CREAM

50. Trends in Ice Cream Costs. Special Bulletin No. 53, Statistical and Accounting Bureau, Int. Assoc. of Ice Cream Mfgrs., Washington, D. C., pp. 1–27, Oct., 1936.

In this bulletin the 1935 ice cream expense dollar is analyzed to show the percentage of the total cost represented by each cost item. The cost of the products used in ice cream represents a higher percentage of the total cost than in recent years because (1) products have increased in cost and (2)

production has increased in volume. The ratio of "Novelty" sales to total ice cream sales shows an increase from 1932 to 1935 in the sale of novelties of 30 per cent. It is stated that this increase in the sale of novelties has affected certain costs and must be considered in any such study. M.J.M.

51. Causes of Defects that Lower the Quality of Our Ice Cream. J. H. Erb, Ice Cream Field *28*, 5, p. 22, Sept., 1936.

The author briefly discusses the most common ice cream defects giving some of the causes for each. W.C.C.

52. Advertising Analysis. Special Bulletin No. 54, Statistical and Accounting Bureau, Intern. Assoc. of Ice Cream Mfgrs., Washington, D. C., Nov., 1936.

The average expenditure for advertising by ice cream manufacturers in 1935 was found to be 2.83 cents per gallon of ice cream. Of the companies reporting, 58 per cent had definite advertising budgets for the following year (1936).

The popularity of the various advertising themes was as follows: quality, 20.6 per cent; food value, 15 per cent; refreshment appeal, 12.2 per cent; health appeal, 11.6 per cent; flavor 10.7 per cent; appetite appeal, 9.1 per cent; confidence in company, 7.5 per cent; seasonal appeal, 7.5 per cent; convenience, 3.5 per cent; economy, 2.3 per cent.

The bulletin contains a detailed analysis of advertising costs and kinds of advertising used in the ice cream industry. M.J.M.

53. Freezers Used by Wholesale Ice Cream Men in New York State. R. L. Gillett and D. H. Foster. Ice Cream Rev. *20*, 4, p. 78, Nov., 1936.

Applications made early in 1936 to the New York State Department of Agriculture and Markets by wholesalers for licenses to manufacture frozen desserts contain information on the number and capacity of "continuous" and "direct expansion" or "batch" freezers.

Of the 218 wholesale plants for which complete figures were available, 23 were equipped with continuous freezers having capacities ranging from 60 to 1,000 gallons per hour. In all but three cases these freezers, representing 10.5 per cent of the plants, had an average annual production of 584,964 gallons per plant in 1935, and made 50.6 per cent of the wholesale ice cream.

In contrast, the 195 plants equipped only with "batch" freezers, included 89.5 per cent of the plants, averaging 67,257 gallons per plant during the year and made 49.4 per cent of the wholesale ice cream.

J.H.E.

54. Vital Facts About a Vital Food. Dairy Industry Committee, Washington, D. C. Released by the Int. Association of Ice Cream Manufacturers, Washington, D. C., pp. 1–24, Nov., 1936.

This is an interesting and instructive booklet about milk and milk products. Economic considerations of milk production and distribution are discussed. The manufacture, food value, and distribution of ice cream and other milk products are briefly reviewed. Data as to the milk production, farmers' income from milk, trends in production of agricultural products, etc., are presented in tabular form. The bulletin can be purchased in quantity at a nominal cost and should prove useful to dairy companies with educational programs for consumers. M.J.M.

55. Formulas for Standardizing Condensed Whole Milk (Before Condensing). C. H. DALE. Ice Cream Field *28*, 5, p. 29, Oct., 1936.

Formulas are given whereby it is possible to adjust the fat and milk solids-not-fat in the product before condensing to that desired in the finished product. W.C.C.

Other articles of interest are numbers 44, 46, 47, 59, and 61.

MILK

56. The Hygienic Quality of Milk in France. G. THIEULIN, National Vet. School of Alfort, France. Le Lait *16*, 154, p. 337, April, 1936.

Lack of demand for milk of good hygienic quality and the fact that the French farmer does not make milk a very important source of income have not operated to improve the quality of milk as rapidly as desired. The writer suggests steps to be taken in the improvement of the hygienic quality of milk in France. This includes a classification of milk into: Milk A, officially controlled sold raw or pasteurized, the milk to be of exceptionally good quality; milk B, ordinary milk but all of it pasteurized milk; Milk C without guarantee, destined for improvement or rejection. Further improvement of the milk requires education of the producer, economic stimulus in the form of payment for milk on the basis of quality, sanitary, medical or veterinary inspection of the herd and barns where the milk is produced, control of pasteurization, testing of the milk as sold etc. A.H.J.

57. The Law of July 2, 1935, on the Sanitation of Market Milk. A. NEVOT, Chief of the Milk Lab. for Sanitary Veterinary Service of the Seine, Paris, France. Le Lait *16*, 154, p. 383, April, 1936.

A discussion is given of the law which classifies milk into grades, A, B, and C. Grade A milk originates from officially controlled herds and may be used raw or pasteurized. Grade B milk is pasteurized milk from officially

controlled herds; and Grade C milk is raw milk from herds not officially controlled. The quality of the milk in the various grades is considered from the chemical, biological and public health view-points. A.H.J.

58. A Contribution to the Study of Buffalo Milk. Production, Properties, Composition and Derived Products. J. Y. Pissarewsky, Laureate of the Academy of Agriculture of France. Le Lait *16*, 155, p. 465, May; 156, p. 592, June; 157, p. 711, July, 1936.

The fact that there are one sixth as many buffalo in the world as there are cows indicate the importance of the buffalo for milk and meat production and as draft animals. While the milk production of many of these buffalo is small, marked increases in milk yield have resulted where effort has been made and where breeding has been done for this purpose. The chemical composition of buffalo milk is given. It is characterized chiefly by its high fat content (5 to 17 per cent, average 7.8) and by its high casein content (3.2 to 9.5 per cent). The high fat and casein content of buffalo milk make it especially valuable for butter and for cheese manufacture. The fat of buffalo milk is very bright colored. Cream does not rise by gravity but cream may be readily obtained by centrifugal separation. The preparation of various cultured milks from buffalo milk is described. Buffalo milk is especially suitable for the preparation of yogurt. A comparison of the chemical composition and physical properties of buffalo, sheep, goat and cow's milk is given. A.H.J.

59. The Surface Tension of Milk. W. Kopaczewski. Le Lait *16*, 154, p. 356, April, 1936.

The author discusses the work of Belle on the same subject. The surface tension of pure milk is a fixed value, established around 53 dynes/cm. Dilution has little effect on the capillary constant of milk. The surface tension of skimmed milk is not much different from that of whole milk, being only very slightly higher. During aging, milk shows a distinct increase in surface tension. The agitation of milk is accompanied by an increase in surface tension. A.H.J.

60. The Hygienic Control of Milk. A Practical and Simple Colorimetric Method. Emilio Zapatero, Dept. of Hygiene and Microbiology of the Faculty of Med. of Valladolid, Spain. Le Lait *16*, 157, p. 689, July and Aug., 1936.

Determination of the contamination of milk with colon organisms was used as a criterion of its hygienic quality. Colon organisms were characterized as follows, a short non-capsulated, non-motile, non-spore-forming bacterium with rounded extremities which was a facultative aerobe and negative to the gram strain, does not liquefy gelatin, ferments lactose yielding gas

and acid, slowly ferments dulcite yielding gas and acid, does not ferment saccharose, coagulates milk, causes a color change with phenol red and with neutral red, produces indol in peptone media, is positive to methyl red, and negative to the Voges-Proskauer reaction. Of the various reactions listed above, fermentation of lactose has been the one most commonly used to determine the presence of *B. coli*. The author, however, prefers the reaction with dulcite. This involves the preparation of a medium 10 grams of Liebig's beef extract, 10 grams of peptone and 5 grams of sodium chloride per liter. This medium is neutralized to litmus with tenth normal sodium carbonate. Five cc. of a one per cent solution of phenol red (dissolved in tenth normal sodium carbonate) is then added. To the proper amount (usually 1 cc.) of the above sterile media is added 1 cc. of a sterile solution of dulcite containing 5 milligrams of dulcite. Sterile physiological saline solution may be added to the culture media. The proper amount of the milk to be tested is then added and the culture tubes kept at 37° C. (98.6° F.) for 24 hours. The appearance of a yellow color indicates the presence of colon organisms and the dilution of the milk at which this yellow color develops indicates the degree of contamination. The use of the dulcite technique instead of the lactose technique appears to be more specific as an indicator of colon organisms originating from fecal material. A.H.L.

61. The Influence of Method of Sterilizing Equipment Upon Development of Oxidized Flavor. A. C. Dahlberg and D. C. Carpenter, New York Agr. Exp. Sta., Geneva, N. Y. Journal of Dairy Science *19*, 8, p. 541, Aug., 1936.

The development of oxidized flavor in pasteurized milk was accelerated by contact of the milk with a clean copper-alloy metal, especially following chlorine sterilization, but this effect became less as more milk continued to pass through the equipment. A.C.D.

62. Marketing Milk in Europe. D. H. Tilson, Aluminum Seal Company. Milk Dealer *25*, 12, p. 142–, Sept., 1936.

The author briefly describes the marketing of milk in Belgium, Denmark, England, Estonia, France, Italy, Netherlands, Norway, Poland, Russia, Scotland, Sweden, and Turkey. The material for this article was obtained by questionnaires sent to the American Consular Service in the above named countries. C.J.B.

Other articles of interest are numbers 39, 40, 44, 46, 48, and 54.

AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

Officers

President	R. R. GRAVES, Washington, D. C.
Vice-President	H. W. GREGORY, LaFayette, Ind.
Secretary-Treasurer	R. B. STOLTZ, Columbus, Ohio
Editor	A. C. DAHLBERG, Geneva, N. Y.
Director	E. G. HOOD, Ottawa, Canada
Director	EARL WEAVER, Stillwater, Oklahoma
Director	M. MORTENSEN, Ames, Iowa
Director	J. A. NELSON, Bozeman, Montana
Director	C. R. GEARHART, State College, Pa.
Director	H. P. DAVIS, Lincoln, Nebraska
Director	H. A. RUEHE, Urbana, Illinois

Officers of Sections

SECTION NO. 1—Dairy Production

Chairman	F. W. ATKESON, Manhattan, Kansas
Vice-Chairman	W. E. KRAUS, Wooster, Ohio
Secretary	I. W. RUPEL, Madison, Wis.

SECTION NO. 2—Dairy Manufacturers

Chairman	P. H. TRACY, Urbana, Illinois
Secretary	J. C. MARQUARDT, Geneva, N. Y.

SECTION NO. 3—Extension

Chairman	C. L. BLACKMAN, Columbus, Ohio
Vice-Chairman	EARL N. SCHULTZ, Ames, Iowa
Secretary	S. J. BROWNELL, Ithaca, N. Y.

Officers of Divisions

Southern	A. H. KUHLMAN, Chairman, Stillwater, Okla.
	T. B. HARRISON, Knoxville, Tenn.
Eastern	A. R. MERRILL, Chairman, Storrs, Conn.
	H. G. LINDQUIST, Vice-Chairman, Amherst, Mass.
	F. J. DOAN, Secretary-Treasurer, State College, Pa.
Western	O. J. HILL, Chairman, Pullman, Wash.
	D. L. FOURT, Secretary, Moscow, Idaho.

The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership shall consist of two kinds: (1) active, (2) associate.

The qualifications for membership in the two classes are as follows: (a) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (b) anyone filling a position of responsibility connected with the dairy industry and who has had a college or University training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a college of Agriculture and who is specializing in dairying. Associate membership it attained by election to membership in a local chapter of *The American Dairy Science Association.*

The dues are $5.00 a year for active membership. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

THE COMPOUNDING OF FINE VANILLA FLAVORING *IS AN ART*

The leadership of Mixevan for quality is the result of over 30 years specialized experience. Its uniform character is achieved through expert knowledge, individual selection of beans, extra development of the bouquet, intricate compounding and special grinding technique . . . *It is the ultimate in fine flavoring for dairy products.*

MARSCHALL

RENNET *and* COLOR

for cheese manufacture

are uniformly strong and pure, hence always dependable. Up-to-date methods and exact control of production insure the quality of Marschall's.

Liberal samples of Marschall preparations for class work or experiment may be had for the asking.

Marschall Dairy Laboratory
INCORPORATED
MADISON WISCONSIN
U.S.A.

THE
MORTENSEN POSITIVE AUTOMATIC SYSTEM OF PASTEURIZATION

A totally enclosed continuous flow method of Pasteurizing milk that meets the requirements of all Health regulations. The holding period is accurately governed by positive automatic gears that cannot be changed. Milk must be held for a minimum of 30 minutes or dumped on the floor.

With either the JUNIOR unit, for plants of small or medium capacities, or the larger unit for plants of large capacities, the milk is not in view from the time it enters the weigh tank until it appears in the bottle. There can be no plant contamination during the processing period.

Made in capacities of from 2,500 to 30,000 pounds per hour in tinned copper or stainless steel and priced as low as is consistent with dependable machinery.

Catalogues will be cheerfully mailed on request, without obligation. Please state the capacities you are interested in.

STANDARD MILK MACHINERY CO.
(INCORPORATED)
LOUISVILLE KENTUCKY

Your advertisement is being read in every State and in 25 Foreign Countries

MODERNIZE
Your Plant *with* a
WESTFALIA
No Foam Separator

- No Foam on Skim or Cream even with elevation of both
- Large Sludge Room, therefore Long Runs without Shut-down
- Higher Skimming Efficiency
- Lower Cost of Operation
- Greater Simplicity of Construction

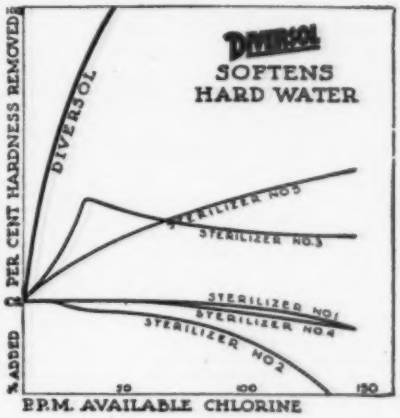
Ask for Catalog.

We also carry a full line of **No Foam Clarifiers and Standard Separators.**

Model MDD

WESTFALIA SEPARATOR CO., INC.
30 Orange St. *Since 1890* **Bloomfield, N. J.**

SOFTENS HARDEST WATER



A comparative test of Diversol and five other dairy sterilizers reveals that Diversol alone completely softens hard water . . . preventing film and scale on equipment . . . insuring against milkstone deposits. Diversol is easy to use . . . dissolves instantly . . . kills bacteria by mere contact . . . and is noncorrosive.

Send **for interesting Bulletin . . . . .**
"Charting a Course to More Efficient Sterilization."

THE DIVERSEY CORPORATION
53 W. Jackson Blvd., CHICAGO, ILLINOIS

DIVERSOL

JOURNAL OF DAIRY SCIENCE

Back Volumes for Sale

Volumes 2 to 14 inclusive may be purchased at $5.00 per volume in complete sets. Prices for single copies or volumes will be quoted by the Secretary.

Back Volumes Wanted

Volumes 1 and 17 are wanted to complete sets and will be purchased for $5.00 per volume.

R. B. STOLTZ,
Secretary-Treasurer
Ohio State University,
Columbus, Ohio.

Your advertisement is being read in every State and in 25 Foreign Countries

For Your Convenience . . .

Spencer Offers This NEW ACCESSORY CATALOG

YOU will find here in conveniently readable form, and well illustrated, a complete catalog of the optical and mechanical accessories and supplies needed by the microscopist.

Objectives, eye-pieces and condensers cover the complete range of magnifications and give suitable corrections for any type of work.

Measuring and counting accessories, including scales, reticules and cross lines to assist the microscopist in tabulating detailed information; camera lucidas for making drawings; vertical illuminators for the examination of opaque specimens; polarizing accessories for studying and measuring optical characteristics; and mechanical stages for convenience in manipulating the specimen are included.

This catalog includes slides, cover-glasses, lens paper, slide labels, immersion oil and slide boxes.

SPENCER LENS COMPANY

Buffalo New York

SPENCER LENS COMPANY
Dept. W-7-2, Buffalo, N. Y.
Please forward this new Spencer Microscope Accessory Catalog.

NAME .....

ADDRESS .....

CITY ..... STATE .....

JOURNAL OF DAIRY SCIENCE

Official Organ of American Dairy Science Association

Agents in Foreign Countries

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Angus & Robertson, Limited, 89–95 Castlereagh Street, Sydney; Stirling & Co., 317 Collins Street, Melbourne.

For Belgium: Henri Lamertin, 58 Rue Coudenberg, Bruxelles.

For the British Empire, except Australia and Canada: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, W. C. 2, London.

For Canada: Wm. Dawson & Son, Ltd., 87 Queen Street East, Toronto.

For China: Commercial Press Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann Co., Inc., Krausenstrasse 38, Berlin NW 19, Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

Subscription price is $6.50 per vollume, postpaid, except in Canada and countries within the Postal Union where the price is $6.00.

The Science Press Printing Company,

Lancaster, Pa., U. S. A.

Your advertisement is being read in every State and in 25 Foreign Countries

"Truly Sanitary" MILK PUMPS

actually exist. Here are the facts. The entire pump is stainless steel and is made in four sizes. Can be entirely taken apart and shaft removed for cleaning in 15 seconds. No packing gland and no lubrication required. Discharge at any desired angle. Equipped with totally enclosed motor, these pumps sell *at a price competitive with brass pumps* of equal capacity.

R. G. WRIGHT CO.

Superior in Stainless Steel

98 East Eagle Street — Buffalo, N. Y.

THE SCIENCE PRESS PRINTING COMPANY

Printers of
Scientific and Educational Journals,
Monograph and Books » » »

LIME AND GREEN STREETS
LANCASTER, PA.

MANTON-GAULIN TWO STAGE HOMOGENIZER

The most Sanitary Homogenizer available, mechanically dependable and unusually efficient. The two stage valve of course for controlled Viscosity and greater uniformity.

The Manton-Gaulin Manufacturing Co.
Incorporated

7 Charlton Street — U. S. A. — EVERETT, MASS.

Your advertisement is being read in every State and in 25 Foreign Countries

DIFCO

Ingredients for the Standard Nutrient Agar

This group of culture media ingredients is recommended for the preparation of nutrient agar according to the formula of "Standard Methods of Milk Analysis" of the American Public Health Association. When used for this purpose neither adjustment of the reaction nor filtration of the medium is required.

Bacto-Beef Extract

Bacto-Beef Extract yields solutions which are sparklingly clear and which have a reaction of pH 6.6 in the concentrations usually employed for culture media.

Bacto-Peptone

Bacto-Peptone is the standard peptone for all general purpose culture media. It is completely and brilliantly soluble and has a reaction of pH 7.0 in a one per cent. concentration.

Bacto-Agar

Bacto-Agar is highly purified and free from extraneous matter. It is prepared in convenient granular form.

Specify "Difco"
THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN

At Your SERVICE

Long years of experience in serving the dairy industry have given the Service Representatives of The J. B. Ford Company unusual ability to be of real assistance to you when troublesome problems arise.

The knowledge and experience of these men are at your service. They are ready to take off their coats and work with you on any problem you may have.

A letter to this office will bring one of them,—and it places you under no obligation whatsoever.

Wyandotte

Order from your Supply Man or write for detailed information.

The J. B. FORD COMPANY **Wyandotte, Michigan**